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EVALUATION OF THE PERFORMANCE OF IODINE-TREATED BIOCIDE FILTERS CHALLENGED WITH BACTERIAL SPORES AND VIRUSES

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14. ABSTRACT

Filter media coated with a cationic resin in triiodide form were challenged by *Bacillus subtilis* spores and MS2 bacteriophage aerosols delivered from a Collison nebulizer through air at 35% RH and 23 C at 14.2 cm/s face velocity. Bacteria were classified and collected in a 6-stage Andersen impactor; serially connected AGI-30 impingers were used in phage experiments. Average bacterial removal efficiency was 99.9991% for untreated (UM) and 99.9994% for treated media (TM); viral removal was 92% for UM and 94% for TM. Microbes collected on filters were extracted into water using a vortex mixer. After correction for effects of extracted iodine, 0.25% of bacteria survived on UM and 0.069% on TM, while 4.2% of phages survived on UM and 2.2% on TM. Increasing concentrations of virus in the nebulizer showed no clear effect from agglomeration.

15. SUBJECT TERMS

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EXECUTIVE SUMMARY

A. OBJECTIVE:

The objective of this project was to evaluate the disinfection capacity of filter media treated with iodinated resin for bacterial spores and viral aerosols. The filter media are being considered as a component of gear protective against bioterrorism and pathogenic airborne biological agents.

B. BACKGROUND:

The increasing threat of biological warfare and the spread of airborne pathogens spurred the public's attention on bioaerosols and the development of protection methods. Filter media combining mechanical filtration and iodine disinfection capacity allow the protection against bioaerosols with a high removal efficiency and lower pressure drop than conventional filter media.

C. SCOPE:

The disinfection capacity of iodine-treated media provided by Air Force Research Laboratory (AFRL) was evaluated with bacterial spores and viral aerosols. Physical removal efficiency and the viability of collected microorganisms on the filter were investigated to determine the effectiveness of the iodine-treated media.

D. METHODOLOGY:

The iodine-treated filter media were challenged by *Bacillus subtilis* spores and MS2 bacteriophage aerosols nebulized from their suspension. The face velocity tested was 14.2 cm/s. A 6-stage Andersen impactor was employed to classify and collect the entering and penetrating bacterial spore aerosols. Serially connected AGI-30 impingers were used in place of the impactor for the collection of viral aerosols. The viability of collected microorganisms on the filter was investigated by enumeration of extracted microorganisms from the filter using a vortexing method. The effect of free iodine in the vortexing solution on viability was factored in the correction of the vortexing experiment results. Different degrees of viral agglomeration were challenged to estimate the shielding effect of agglomeration.

E. TEST DESCRIPTION:

Various filter media were tested against bacterial spores and viral aerosols. Tests were conducted at low humidity (35 $\pm$ 5%) and room temperature (23 $\pm$ 2 °C). After 10 hrs of filtration experiments, filters retrieved from the experimental apparatus were subject to the vortexing experiment with sterile deionized water to investigate the viability of collected microorganisms on the filter. To generate the different degree of agglomerated particles, the concentration of virus in the nebulizer was varied by changing the amount of virus stock.

F. RESULTS:

Both iodine-treated (JT-70-20XP-10T-100) and untreated (JT-70-20XP-100) filters tested for bacterial spores aerosols at low relative humidity (35 ± 5%) and room temperature (23 ± 2 °C) presented high collection efficiencies, which were 99.9994 ± 0.0008 % and 99.9991 ± 0.0012 %, respectively. The initial pressure drop of the evaluated filter was around 3.0 ~ 3.4 in H_2O, and the variance in the pressure drop during the 10 hrs of experiment was almost negligible. The survival fractions, which are defined as the ratio of the extracted microorganisms to the collected microorganisms on the filter were $6.9 \times 10^{-4} \pm 1.6 \times 10^{-4}$ and $2.5 \times 10^{-3} \pm 1.4 \times 10^{-3}$ for the iodine-treated and the untreated filter, respectively.

Different filter media (polyester–cotton 125 gsm iodinated resin and polyester–cotton 300 gsm iodinated resin) were supplied by AFRL for the viral aerosols. The collection efficiency of both iodine-treated and untreated filters for viral aerosols at the same humidity and temperature was 94 ± 3% and 92 ± 2%, respectively. The pressure drop was almost constant at 0.2 in H_2O during the 10 hrs of experiment. The survival fraction was $2.2 \times 10^{-2} \pm 8 \times 10^{-3}$ and $4 \times 10^{-2} \pm 3 \times 10^{-2}$ for the iodine-treated and untreated filter, respectively.

No significant difference in the survival fraction of low and high degrees of agglomerated particles was observed implying negligible shielding effect of agglomerated particles studied in this research.

G. CONCLUSIONS:

The novel technology combining physical collection mechanism of filter media and chemical disinfection characteristics of iodine presented high removal efficiency, a lower pressure drop and less viability of collected microorganisms on iodine-treated filter than untreated filter for bacterial spore aerosols. Different filter media tested for viral aerosols showed collection efficiency higher than 90% but lower than that for bacterial spores with almost constant pressure drop. The average survival fraction of iodine-treated filter for viral particles was lower than that of untreated filter. However, they are not statistically different due to the large variation in the results. A higher degree of viral agglomeration did not exhibit any significant shielding effect.

H. RECOMMENDATIONS:

Further studies are needed to determine the effectiveness of iodine-treated filter media in the real world under versatile scenarios. The interference of dust loading should be examined and the presence of materials that may react with the active sites of the filter media should be evaluated. Use of thiosulfate solution as vortexing solution for excluding the effect of free iodine and characterizing the agglomerated particles are recommended for future experiments.

PREFACE

This report was prepared by the Aerosol and Particulate Research Laboratory, Department of Environmental Engineering Sciences, University of Florida, Gainesville, FL 32611-6450, under Contract Number FA8651-05-C-0136 for the Air Force Research Laboratory (AFRL/MLQ), 139 Barnes Drive, Tyndall AFB, FL 32401-5323.

This is a final report being submitted to AFRL/MLQ. It describes work that was performed from April 05 to April 06. The Air Force Technical Program monitor was Dr. Joseph D. Wander. We greatly appreciate the assistance of Triosyn Corp., Dr. Dale Lundgren, Dr. Samuel Farrah, and Dr. Jean Andino at the University of Florida, for their valuable advices. We also thank Katherine M. Wysocki from Department of Chemical Engineering at University of Florida for her assistance in the laboratory.

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1 INTRODUCTION

1.1 Objective

The goal of this project was to evaluate the disinfection capacity of iodine-treated filter media developed by Triosyn Corp. for bacterial spores and viral aerosols. Collection efficiency and viability of collected microorganisms on the iodine-treated filter were estimated. The ultimate goal is to use the iodinated resin filter as a protective gear against bioterrorism and pathogenic airborne biological agents.

1.2 Background

1.2.1 Biological threat

Increasing concerns on bioterrorism after the anthrax attack on September 18, 2001, and the spread of airborne pathogens such as the Severe Acute Respiratory Syndrome (SARS) virus have attracted the public's attention on bioaerosols and protection methods. Biological agents have been used throughout the history as a weapon. In the 6th century B.C., Assyrians poisoned the wells of their enemies with rye ergot. In 1995, Aum Shinrikyo attempted on several occasions to release biological agents such as anthrax, *botulinum* toxin and ebola in aerosol form. Biological warfare agents can be made even by small groups and terrorist organizations because the production of bacteria, massive toxins and virulent strains of virus is easy and inexpensive. Pathogens are far more destructive than chemical weapons; a few kilograms of anthrax optimally delivered can kill as many people as a Hiroshima-size nuclear bomb [1]. The spread of biological agents is silent, having the characteristics of invisible, odorless, and tasteless, and almost undetected until symptoms are developed by infected people. They can be spread widely throughout a city or region, in contrast to chemical agents, which spread in a downwind area near the point of release [2]. *Bacillus anthracis*, one of the agents of concern listed by Centers for Disease Control and Prevention (CDC), was used as a bioterrorism weapon in 2001 resulting in five deaths out of the 11 people known to have inhaled it. Approximately 60 million dollars were spent to provide medical treatment to affected workers and to test and clean up the facility. It also resulted in the launching of "*Public Health Security and Bioterrorism Preparation and Response Act of 2002*" by the US government [3].

The spread of airborne pathogens is another emerging problem increasing the public's awareness of bioaerosols. SARS, a viral respiratory illness caused by a corona virus for which there is no vaccine and no cure, was first reported in Asia in February 2003 and spread to more than two dozen countries in North America, South America, Europe, and Asia over the following few months. Seven hundred and seventy-four people died amongst the total of 8,098 worldwide infected with SARS during the 2003 outbreak. It is suspected that SARS spreads through droplets generated from sneezing or coughing by infected persons, which subsequently deposit on the mucous membranes of the mouth, nose or eyes of persons who are nearby [4]. Diseases transmitted by the respiratory route include influenza, community acquired pneumonia, *Legionella*, rhinovirus, measles, meningitis and tuberculosis, in addition to many not known to humans [5].

1.2.2 Bioaerosols

Even though interest in bioaerosols has recently been highlighted, bioaerosols have been present in the environment from the origin of mankind in both indoor and outdoor air. Bioaerosols are aerosols of biological origin including viable bacteria, viruses, fungi and algae, as well as such nonviable materials as dust mites, pollen, endotoxins, mycotoxins and various allergens [6]. The diameter of bioaerosols ranges from smaller than 0.1 μm to 100 μm. Although the size of a single bacterium is commonly around 1 μm with various shapes such as spheres (cocci), rods (bacilli) or spirals, they present in larger sizes in the ambient air as aggregates. Larger bioaerosols are influenced by gravitational force and are removed from air by settling in a short period of time. In contrast, smaller bioaerosols can remain in the air for a prolonged period of time.

Bioaerosols are associated with a wide range of adverse health effects such as allergy, organic toxic syndrome, asthma and other respiratory illnesses. Bioaerosols must be viable to be infectious, but viability is not a prerequisite to allergenic and toxic effects [7]. Non-viable bioaerosols can also cause such allergic reactions as hay fever, rhinitis, and asthma by contact and inhalation [8]. The various diseases transmitted by bioaerosols include tuberculosis, mumps, measles, rubella, pneumonia, meningitis, Legionnaires', influenza etc. [9]. Biological agents are also correlated with such building-related illnesses (BRIs) as Legionnaires' disease and aspergillosis [10]. Airborne transmission of respiratory diseases is classified into two groups: communicable and non-communicable. Communicable diseases can transmit between human hosts, while non-communicable diseases come only from the environment due to fungal or actinomycete spores and environmental or agricultural bacteria [11].

Most terrestrial surfaces exposed to air movement can be potential sources of bioaerosols. Microorganisms in natural waters as well as anthropogenic water remain airborne after evaporation of the liquid resulting from rain, splashes, or bubbling processes. The growth and multiplying of microorganisms in a new environment of engineered systems such as humidifiers, evaporative air coolers, cooling coil drain pans, and condensation on ductwork insulation can result in an amplification of microorganisms to unhealthy levels [11]. Therefore, the heating, ventilation and air conditioning (HVAC) system of a building could be a major source of bioaerosols indoors [7]. In occupational environments where organic materials such as plants, hay, cotton, metalworking fluids organic waste, and wastewater are handled, workers are exposed to high concentrations of bioaerosols. Through sneezing and coughing, humans are also an important sources of bioaerosols—a single sneeze can generate a hundred thousand bioaerosols. A single cough produces only one percent of this amount, but occurs 10 times more frequently than sneezes [11]. Thousands of droplets approximately 1 to 10 μm in diameter and containing viable microorganisms released by a person will quickly evaporate to droplet nuclei. For instance, the evaporation time of a 12-μm droplet is only 0.02 s. Droplet nuclei remain suspended in air for a long time and travel considerable distances by attaching to aerosols existing in air. Especially, respiratory viruses such as influenza virus appear to be spread mainly by droplet nuclei [12, 13]. Due to droplet encasement, virus infectivity is shielded from drying,

sunlight, and temperature compared to an isolated airborne virus [14]. In indoor environments, microorganisms are also free from factors inducing their destruction, thus resulting in longer survival of airborne microbes. Direct sunlight has the potential to kill microorganisms since it contains a lethal level of ultraviolet radiation. Oxygen and air pollutants may also cause destruction of microbes. A study on the viability loss of airborne microbes revealed that in the absence of sunlight bacteria decay faster in air than viruses since bacteria depend more on moisture for their survival than viruses do [11].

1.2.3 Filtration

The advantages of simplicity, versatility, and economical collection of aerosol particles make filtration the most common method for aerosol removal, and it is used extensively in HVAC systems as well as in respiratory protection [15]. The ability of filters to collect particles is described by their *collection efficiency*, defined as the fraction of impinging particles retained in the filter, and *pressure drop*, which is related to energy cost. The three common mechanisms associated with filtration collection are interception, inertial impaction and diffusion. Large particles unable to quickly adjust themselves to the changing gas streamline near the fiber will cross the streamline and hit the fiber by inertial impaction. In contrast, small particles encounter the fiber due to Brownian motion. When particles follow the streamline perfectly (*i.e.*, inertia, settling and Brownian motion are all negligible), particles collect by interception on the filter fiber due to its finite size [15].

The aerodynamic particle size is not the only factor to be considered in the collection of bioaerosols. The physical properties of microorganisms—including shapes of aerosols and surface structure—are also important factors in collection on the filters. Qian et al. [16] reported that penetration by polystyrene latex spheres was higher than that of *M. chelonae*, a rod-shaped bacterium of similar aerodynamic size. A similar study reported that penetration by rod-shaped organisms was lower than that by spherical organisms [17]. In evaluating the collection efficiency of various filter media impregnated with potassium chloride (KCl) particles and fungal spores, Jankowska et al [18] reported that the collection efficiency of fungal spores was slightly lower than that of KCl particles of the same aerodynamic size due to breakup of the aggregates. The aggregation of microorganisms also affects the resistance of microorganisms to inactivation. The survival curve of viral particles irradiated with ultraviolet light was strongly dependent on the degree of aggregation among the viral particles [19].

There have been numerous studies conducted to evaluate various air filter media for the removal of bioaerosols, *e.g.*, surgical respirators. The role of respiratory protection devices against TB in health care settings has been reviewed, and it was reported that surgical masks are not adequate to remove bioaerosols in the submicron size range [20–24].

A high-efficiency particulate air filter (HEPA) has high retention of bioaerosols, capturing 99.97 % at the nominal most-penetrating aerosol size of 0.3 μm [25]. The main problem in conventional filtration is the high maintenance associated with a large pressure drop. Furthermore, there are concerns about the growth of microorganisms previously

collected on the filter, which may result in the release of byproducts and reentrainment. Under suitable growth conditions such as sufficient nutrient, and proper humidity and temperature, collected microorganisms can proliferate and grow, causing illness and allergies by reentrainment into the air [8]. Even though HVAC prevents the contamination of indoor air from environmental bacteria and spores entering from outdoors, once their growth occurs in the system, they appear in returned air at a higher level than in the outdoor air [11]. It has been shown that fibrous building materials—including insulation substances and ceiling tiles—serve as nutrients for the growth of microorganisms under sufficient relative humidity [26, 27].

Research about the effect of air filter media on the viability of bacteria showed that the fiber materials did not have an inhibitory effect on the growth of microorganisms and the survival of microorganisms even if they did not grow [28]. Sensitive cells lost their viability in less than three days after collection, but resistant bacteria such as *B. subtilis* spores can retain viability on the filter for a much longer time [29].

Microorganisms surviving on the filter can reentrain into air passing through the filter medium. This has been reported in several studies [24, 29–31]. A study of the reaerolization of bacteria and solid particles from N95 respirators observed that reaerosolization of larger particles into air is significant at the high reentrainment velocities corresponding to violent sneezing and coughing at a low relative humidity level of 22 % [30]. The reentrainment of fungal spores was higher than that of KCl particles due to disaggregation of fungal spores. Moreover, the rate is different among various fungal spore species depending on the surface structure [18].

Deactivation of previously collected microorganisms is important for two reasons: one is to prevent contamination of ambient air by reentrained microorganisms and the other is to extend the lifetime of filtration systems by preventing proliferation of microorganisms in the filter. Therefore, antimicrobial treatment intended to destroy or inhibit the growth of microorganisms on air filters has been used for several years [32].

1.2.4 Iodine as a disinfectant

Elemental halogens (Cl_2, Br_2, I_2, etc.) exist as diatomic molecules and form salts with sodium and other metals [1]. Chlorine and iodine have a rich history of use as antimicrobial agents. Chlorine is the most commonly used disinfectant in water treatment among diatomic halogens due to its relatively low cost. However, unacceptable residual levels of chlorine are a possible disadvantage of using chlorine as a water disinfectant. Iodine is superior to chlorine due to the greater chemical stability of the product and less reactivity with organic nitrogenous contaminants in water [33]. Moreover, iodine is very stable in water over a wide *p*H range (6–8) and has low solubility in water. It has been used by the military and by developing countries in such emergency or temporary uses as portable water purification. However, continuous consumption of iodine-treated water is not recommended due to its adverse health effect. In aqueous solution, iodine may exist as various species since iodine can form compounds in all oxidation states from –1 to +7. The overall reaction of iodine in water starts from hydrolysis to form hypoiodous acid (HOI) as

shown in Eq. (1). Hypoiodous acid then disproportionates to iodate (IO_3^-) and iodide (I^-)as depicted in Eq. (2). Eq. (3) presents the overall reaction by combing these two reactions. According to this equation, iodine molecules are significant in acidic conditions. In neutral and basic solution, iodide and triiodide coexist as shown in Eq. (4). At high *p*H (>10), HOI dissociates to hypoiodite ion (OI^-) and hydrogen ion (H^+) as shown in Eq. (5) [33].

$$3I_2 + 3H_2O \leftrightarrows 3I^- + 3HOI + 3H^+ \quad (1)$$

$$3HOI \leftrightarrows 2I^- + IO_3^- + 3H^+ \quad (2)$$

$$3I_2 + 3H_2O \leftrightarrows 5I^- + IO_3^- + 6H^+ \quad (3)$$

$$I_2 + I^- \leftrightarrows I_3^- \quad (4)$$

$$HOI \leftrightarrows H^+ + OI^- \quad (5)$$

Although molecular iodine has disinfection properties, the most effective form of disinfectant is hypoiodous acid. As shown in Eq. (5), however, hypoiodous acid is not stable at high *p*H. Hence, molecular iodine is more important in the inactivation of microorganisms if time is sufficient to penetrate the cell wall [34]. Both disproportioniated species (IO_3^- and I^-) are not considered to be virucidal [35]. It is speculated that iodine molecules penetrate the cell wall of microorganisms and react with N–H, S–H and phenolic groups, resulting in the disruption of normal function of amino acids [36, 37]. As a consequence, iodine is bactericidal but it does not inactivate either infectious ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) [38]. Meanwhile, study of the sporicidal effect of iodine with *Bacillus metiens* spores showed that the decrease of germicidal activity is due to increased iodine decomposition [39]. Studies on the bactericidal and virucidal properties of iodine reported that the bactericidal effect is complete within 1 min of contact [40]. Generally, iodine inactivation is effective in clean water, at higher *p*H, at higher temperature and at higher iodine dose.

When using iodine as the disinfectant for such fluids as water and air, care should be exercised due to the risk of iodine vapor ingestion and concern for hypothyroidism. Iodine vapor is irritating to mucous membranes and has adverse health effects on the respiratory system [41].

1.2.5 Iodinated resin filter media

Iodinated resins have been developed to provide release-on-demand of iodine residuals for disinfection. Iodine can be attached to a quaternary ammonium strong base anion resin in the form of triiodide (I_3^-) and pentaiodide (I_5^-) anions. Iodinated resins are positively charged while microorganisms are negatively charged, leading to attraction of the microorganisms to the resin and transfer of the I_2 molecules upon contact. Even though pentaiodide resins cause 2–3 log scales higher reduction of microorganisms, the triiodide form is preferred because minimal release of I_2 is desired to maximize the life of filter media [42]. Iodinated resin filters have been used in the microbial check valve (MCV) for potable water disinfection since the inception of the space shuttle program. Research on the

disinfection property of iodinated resins at unit gravity and under microgravity reported that iodinated resins are more effective at unit gravity than microgravity [43]. Many studies on the disinfection capacity of iodinated-resin filters for treatment of bacteria and virus in water were conducted at least three decades ago and reported high disinfection capacities, over 99.99% [43–46]. However, very few studies on the disinfection capacity of iodine resin filter for air treatment have been conducted. Iodine-treated filter media are being developed by Triosyn Corp. Their novel filter combines the advantages of mechanical filtration with the disinfection property of iodine to treat air contaminants. Triosyn® filters are intended to reduce health risk by responding to electrostatic forces and releasing iodine, which binds to microorganisms, on demand. Iodine so released deactivates microorganisms by oxidizing cell components and iodinating cell proteins [1] as discussed above.

1.3 Scope

In our previous study [47], the high biological removal efficiency was compared to the physical capture efficiency of an iodine-treated filter. The filter was demonstrated to have a significantly smaller pressure drop than that of glass fiber HEPA filters. The test microorganisms were *Escherichia coli* and *Micrococcus luteus*, which were frequently used as representative bioaerosols. To ensure the reliability of iodine-treated filters as protective gear against airborne pathogens and biological agents, we concluded that studies on more-resistant microorganisms and the smallest size range of bioaerosols are needed. Further, we identified investigating the viability of microorganisms collected on the filter as a critical step to prove the disinfection capacity of the iodine-treated filter.

Another important parameter to be considered in evaluating iodine-treated filters is the state of agglomeration of bioaerosols. If collected bioaerosols are agglomerated, microbial particles in the core surrounded by the outer layers of microbial particles will be shielded or prevented from contact with disinfection materials on the filter. It is well known that bioaerosols are dispersed over a wide size range and that various sizes of agglomerated microorganisms are present in the air [6]. Therefore, consideration of this factor is needed in the evaluation of this biocidal filtration system. This report describes the performance and interpretation of experiments conducted to evaluate the above parameters.

2 APPROACH

Two experimental procedures were used in this study to evaluate the disinfection capacity of iodine-treated filter media as a protective device for biological particles. The first procedure involved a filtration system to measure the particle and viable removal efficiency of filter media. The second procedure was the use of vortexing experiments to investigate the viability of microorganisms collected on the filter. Different degrees of bioaerosol agglomeration were generated on the filter by changing the concentration of organisms in the reservoir of the Collison nebulizer to evaluate the shielding effect of bioaerosols. Specifically,

1. The efficiency with which iodine-treated filters remove bacterial spore aerosols and viral aerosols was evaluated.
2. The viability of microorganisms collected on the iodine-treated filter was investigated.
3. The shielding effect of bioaerosols depending on the degree of agglomeration was investigated.

3 EXPERIMENTAL

3.1 Test Microorganisms

Bacillus subtilis spores supplied by the Department of Microbiology and Cell Sciences at University of Florida and MS2 bacteriophage (*Escherichia coli* bacteriophage ATCC® 15597-B1™) were tested. *B. subtilis* is a Gram-positive, non-pathogenic rod-shaped bacterium 2.0–3.0 μm in length and 0.7–0.8 μm in width [1]. *B. subtilis* spores are a commonly used surrogate for the *B. anthracis* spores used as a bioterrorism agent in 2001.

MS2 bacteriophage (MS2) has been used as a surrogate for pathogenic viruses in many studies [28, 48, 49]. It infects and replicates in male *E. coli* C3000 with sex pili. MS2 is a single-stranded RNA, un-enveloped and icosahedron-shaped with single-unit diameter around 24 nm. Human pathogenic enteroviruses such as poliovirus have similar physical characteristics [50]. The important consideration in the selection of a model virus for evaluation of disinfection capacity of antimicrobial agents is the viral resistance to agents, because the inactivation resistance of viruses varies. Berg et al. [35] studied the effects of the virucidal properties of iodine molecules with several enteroviruses and reported that coxsackievirus strains are more resistant to iodine inactivation than poliovirus type 1 or echovirus type 7. Another study reported [51] that poliovirus type 1 and echovirus type 1 are more resistant to iodine inactivation than is hepatitis A. The enteroviruses are generally considered more resistant to halogenation than enteric viruses. Iodine inactivation is accomplished through interruption of the viral protein coat. Since both enterovirus and MS2 lack lipid components, resistance of MS2 to halogenation is considered similar to that of enteroviruses such as poliovirus, coxsackievirus, and hepatitis A virus.

3.2 Bacterial Aerosols Experiment

3.2.1 Spore production and purification

Sporulation is the transformation of vegetative cells into spores when they encounter an extremely harsh environment such as lack of nutrient and high temperature. Spores can endure the extreme condition and revert to vegetative cells when proper conditions for cell growth reappear in the environment. In this study, the African violet method (African violet soil, 77.0 g; Na_2CO_3, 0.2 g; distilled water, 200.0 mL) suggested by The American Type Culture Collection [52] was chosen for sporulation. The nutrient agar was made of 25% extract of soil autoclaved and mixed with 75% sterile distilled water. *B. subtilis* was inoculated in the African violet agar slant and incubated at 36 °C for one week to produce spores 0.8~1.2 μm in length, of either spherical or ellipsoidal shape [53]. After spore production, bacterial growth was harvested into 2 mL of sterile distilled water and poured into a sterile glass tube. The glass tube containing the spore suspension was heated in a water bath at 80 °C for 30 mins to kill vegetative cells. After cooling, the spore suspension was diluted with 5 mL sterile distilled water and centrifuged at 3500 rpm for 5 mins. Separated cell debris was then removed in the supernatant. This process was repeated two more times and the spores were resuspended in 5 mL sterile distilled water. After this

purification process, the spore suspension was stored in a refrigerator at 4 °C before experimentation. Microscopic observation of the spore suspension after applying the malachite green spore-staining technique [54] demonstrated that endospores were the principal constituent, with minute amounts of cell debris.

3.2.2 Aerosol generation and environmental conditions

The experimental system for bioaerosol generation is shown in Figure 3.1. Iodine-treated (JT-70-20XP-10T-100) and untreated (JT-70-20XP-100) filters were supplied by AFRL. A six-jet Collison nebulizer (Model # CN25, BGI Inc.) was used to aerosolize microbial suspensions. The flow rate was 7 Lpm. Spore suspensions made by dispersing 0.1 mL of purified spore suspension in 150 mL sterile distilled water were delivered into the nebulizer reservoir. The aerosolized suspension was diluted with filtered compressed air in a 2.3-L glass dilution chamber. A flow rate of 15 Lpm—which corresponds to the face velocity (14.2 cm/s) used in Triosyn's tests—was used and controlled by a calibrated rotameter. The experiments were conducted for 10 hrs at low humidity (35 ± 5 %) and room temperature (23 ± 2 °C).

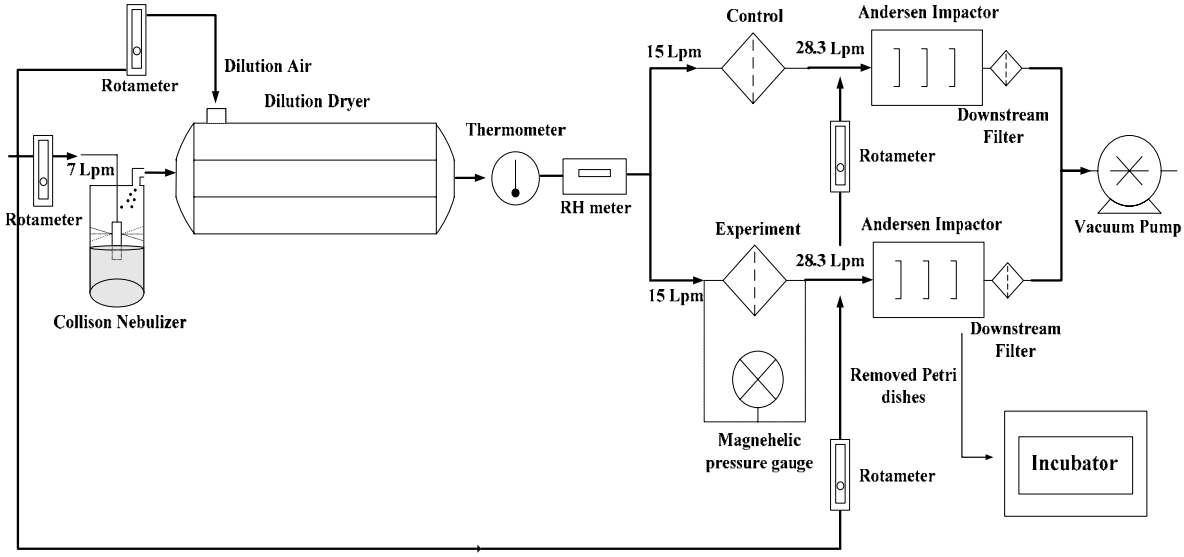


Figure 3.1. Experiment setup for bacterial aerosol system

3.2.3 Andersen six-stage impactor

An Andersen six-stage viable impactor (#10-820, Thermo Electron Corp.) was used to classify generated bacterial particles and those that penetrate the test filter. The Andersen impactor is designed to collect viable microbial particles onto dishes containing 27 mL agar inserted at each stage under an aluminum plate with 400 precisely drilled jet orifices. The 27-mL volume is recommended by the manufacturer to ensure the proper jet-to-plate

distance at the designated 28.3 Lpm flow rate. The orifice diameter of each plate decreases successively from the first stage (1.81 mm) to the sixth stage (0.25 mm) to collect particle size ranges from 20 μm on the first stage to 0.65 μm on the sixth stage. Table 3.1 lists the jet diameter and the corresponding particle size range for each stage. After sampling, glass Petri dishes filled with either nutrient or tryptic soy agar were removed from the impactor, reversed, and incubated for 24–36 hrs before enumeration of microorganism growth. To prevent contamination of ambient air a glass fiber HEPA filter (47 mm, Millipore) was placed downstream to capture spores, if any, not collected by the sampler. However, the cut size of the last stage (0.65 μm) is much smaller than the nominal size of the spore (1.0 μm), and therefore no collection is expected.

Table 3.1. Jet diameter and particle size range of Andersen impactor for each stage when operated at 28.3 Lpm

Stage	1	2	3	4	5	6
Jet Diameter (mm)	1.81	0.91	0.71	0.53	0.34	0.25
Particle size range (μm)	> 7.0	4.7–7.0	3.3–4.7	2.1–3.3	1.1–2.1	0.65–1.1

The experiments for both iodine-treated filters and untreated filters were conducted for 10 hrs. The removal efficiency of each filter was calculated by enumerating micro-organism growth in agar plates of two impactors, one downstream of the test filter and the other, which has no test filter, for baseline. The collection efficiency (η) was determined as:

$$\eta \ (\%) = \left(1 - \frac{N_{penetration}}{N_{total}}\right) \times 100 \qquad (6)$$

where N_{total} is the total number of entering bioaerosols and $N_{penetration}$ is the number of microorganisms downstream.

Experiments were started by collecting bacterial aerosols at all six stages with no test filter for 5 mins as the baseline. Bacterial aerosols collected during the final 5 minutes also served as a baseline. The average number of colony forming units (CFUs) in both measurements were used in determining the feed aerosol concentration. After the first 5 min, the test filter was introduced into the experiment. The impactor downstream of the test filter contained only the sixth-stage agar plate because penetration of the bacterial aerosol through the test filter was expected to be low. The agar plate was replaced with a fresh one every 20 mins for 2 hrs to avoid overloading and dehydration of the agar. Five 2-hr trials were conducted, so the total evaluation time for each filter was 10 hrs, 2 hrs longer than the standard working shift of 8 hrs.

Agar plates containing more than 300 colonies were counted by the positive hole method. This method is essentially a count of the jets and the conversion of this count into a particle count by the use of a positive hole conversion table. Its principle is that as the

number of viable particles impinging on a given plate increases, the probability that the next particle will go into an empty hole decreases. The values of the positive hole conversion table are calculated from the basic formula

$$\mathrm{P_r} = N\left[\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + ... \frac{1}{N-r+1}\right] \tag{7}$$

In which P_r is the expected number of viable particles to produce r positive holes and N is the total number of holes per stage, 400 [55].

3.2.4 Viability of spores on the filter

After 10 hrs of experiments the filter medium were retrieved from the filter holder in the experimental apparatus to investigate the viability of the microorganisms collected on the filter. The filter was removed with a sterile forceps and placed in a 200-mL beaker. Forty mL sterile distilled water was added. Previous studies on extraction of bacteria from filter media showed that vortexing is a more efficient method for recovery than mechanical shaking and agitation in the ultrasonic bath [29]. Hence, the beaker was agitated with a vortex mixer (Model # M16715, Barnstead) for designated times. After 1 min vortexing, 1 mL of sample was withdrawn for measuring the viability of extracted microorganisms in the original solution and an additional 1 mL was withdrawn for dilution. This procedure was repeated after 2, 3, 5, and 10 mins of vortexing time without changing the solution. The count of extracted microorganisms, C_E, was determined as:

$$C_E = \frac{cfu}{10^{-n}} \times \frac{V_1}{V_2} \tag{8}$$

where *cfu* is the colony forming units, V_1 is the volume of extraction fluid, V_2 is the volume of diluted suspension spread on agar plate and n is the dilution factor.

The total viability of extracted microorganisms was calculated by averaging the number of viable microorganisms at all vortexing times. To compare the results of iodine-treated filters with the untreated filters, the *viable fraction* [29], which is defined as the ratio of the viable count in the extract to the total collected on the filter for 10 hrs, was used.

In aqueous solution, the resin surfaces may release iodine molecules that can deactivate microorganisms. This reaction raises concern that microorganism can lose their viability in the extract solution due to the free iodine residual, rather than on the filter. If true, this phenomenon will lead to an erroneous conclusion that a collected microorganism was deactivated on the iodine-treated filter. To investigate this possibility, the solution after vortexing the clean iodine-treated filter was inoculated with a known spore concentration. The spore concentration was then measured to determine the effects. The vortexing effect on the viability of microorganisms was also excluded by vortexing a known concentration of spore suspension at each designated vortexing time.

3.3 Viral Aerosols Experiment

New filter media different from those used in bacterial experiments were supplied by AFRL. Two types of iodine-treated filter, polyester–cotton coated with 125 g/m3 (gsm) Triosyn™ and polyester–cotton coated with 300 gsm Triosyn™ were tested. An untreated filter of the same thickness as the polyester–cotton 125 gsm Triosyn™ was also examined.

3.3.1 Virus stock and host cell preparation

A virus stock was harvested by scraping soft, top agar that had confluent lysis into 10 mL of tryptone broth. Equal volumes of broth containing virus and top agar were dispensed into two centrifuge tubes and centrifuged at 4500 rpm for 20 minutes. The supernatants in the tube were passed through a 0.22-μm pore-size filter and the filtrate was used as the MS2 bacteriophage stock suspension. This suspension was stored in a refrigerator at 4 °C for periods of no longer than 2 months before use in the experiments.

The host cell of MS2, *E. coli* C3000, was spread evenly over the entire slant surface by using a sterile inoculating loop. After incubation overnight at 36.5 ± 2 °C, the *E. coli* 3000 culture was inoculated into 5 mL of tryptone broth using a sterile inoculating loop and incubated for 16 hrs at 36.5 ± 2 °C. A 1.5-mL portion of the 16-hr culture was transferred into 30 mL of tryptone broth in a 125-mL flask and incubated for 4 hrs at 36.5 ± 2 °C with gentle shaking.

3.3.2 Aerosol generation and environmental conditions

The experimental set-up for testing viral aerosols is shown in Figure 3.2. Three concentrations of viral suspension were used in the nebulizer reservoir to generate three

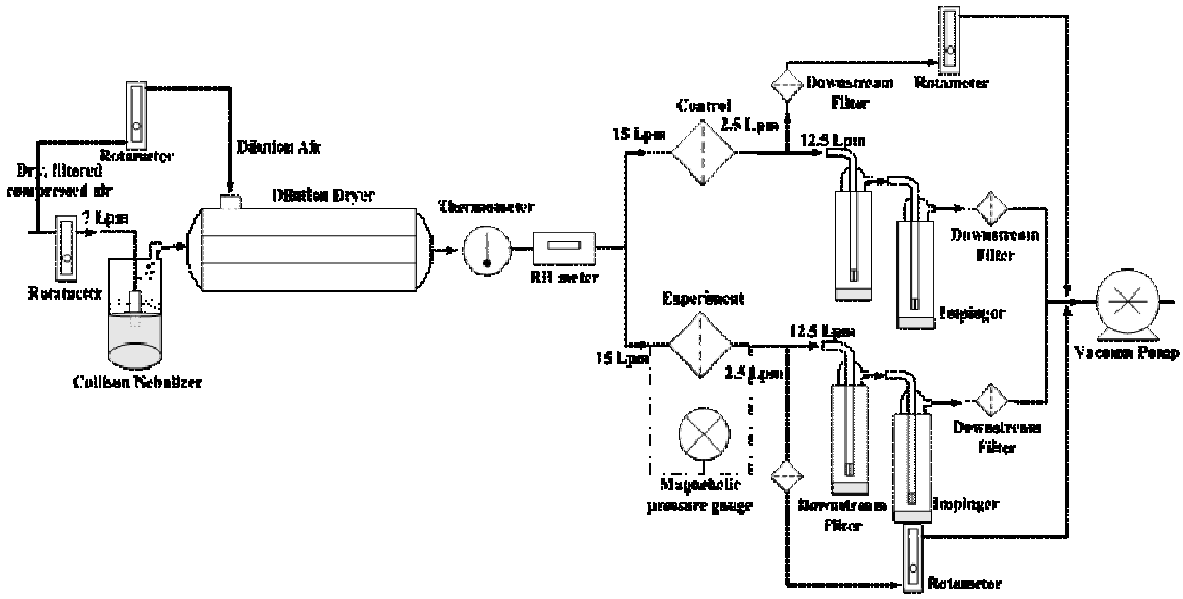


Figure 3.2. Experiment setup for viral aerosol system

different agglomerated sizes of bioaerosols. These were prepared by inoculating 0.1 mL, 0.5 mL, and 1 mL, respectively, of virus stock into 150 mL of sterile distilled water. Seven Lpm of dry, filtered, compressed air was passed though a six-jet Collison nebulizer (Model # CN25, BGI Inc.) to aerosolize the viral suspension. The aerosolized particles were dried with filtered compressed air in a 2.3-L glass dilution dryer. The same flowrate of 15 Lpm as that used in the bacterial experiment was controlled by using calibrated rotameters. Penetrating and reaerosolized viral particles were collected in the downstream filter (Millipore glass fiber filter, Lot # H3NN53241).

The relative humidity for viral aerosol experiments was maintained at 35 + 5 % due to the resistance characteristics of MS2 below 40 % RH [56]. Experiments were carried out for 10 hrs at room temperature (23 + 2 °C) at ambient pressure.

3.3.3 AGI-30 Impinger

The AGI-30 impinger (Ace Glass Inc., Vineland, N.J.), which uses an aqueous solution as the collection medium, is an all-glass impinger with a 30-mm jet-to-plate distance. Air is drawn by vacuum and particles are collected by impingement into the liquid. The 20-mL volume of aqueous collection medium lessens damage to viable cells during sampling operations, such as dehydration and impaction by direct contact with the base of the sampler. A suitable dilution of collection liquid provides adequate counts of micro-organisms. Moreover, the liquid-collected microorganisms can be used with a variety of analytical methods, including culture, microscopy, immunoassay, flow cytometry and molecular methods. Retention of infectivity of MS2 bacteriophage in sterile deionized water is more than 80 % after 8 hrs at room temperature (25 °C) [57]. Therefore, sterile deionized water was used as the collection medium.

Since the cutoff diameter is 0.3 μm at a 12.5-Lpm flow rate [6], low collection efficiency is expected for viral particles. Some losses of collected bioaerosols may also occur through reaerosolization into the airstreams, and through collection stresses, *e.g.*, shear force, that reduces bioaerosol viability. However, there is no known biosampler that has a high collection efficiency of viral particles. Furthermore, such losses are expected not to affect this study because the test results are compared with the baseline, for which the flow line is identical except that the test filter is absent.

The double-agar-layer assay method described by Adams [58] was used for plaque assays. Three mL of melted tryptone top agar was added to 16×150-mm screw-capped test tubes and kept in a water bath at 44.5 $\pm$ 1 °C to avoid premature solidification of the agar. A 1-mL sample aliquot was added to a test tube and 1 mL of tryptone broth was added to another test tube as a control. The host culture (0.l mL) was added to each of the test tubes and control tubes. Each mixture was immediately poured over the bottom agar layer of a Petri dish and the dish was tilted to spread the suspension evenly over the surface of the bottom agar. It was placed onto a level surface to allow the agar to solidify. After hardening, the Petri dishes were inverted and incubated at 36.5 $\pm$ 2 °C overnight and examined for plaques on the following day.

3.3.4 Removal efficiency

Viral aerosols were collected in the impingers every 30 mins for 10 hrs. The removal efficiency was determined by counting plaques on each Petri dish of both impingers, one downstream of the test filter and the other, which has no test filter, for a baseline. The removal efficiency (η) was calculated according to Eq. (6). In calculating viral concentration, a dilution factor was used corresponding to the degree of transfer of the impinger solution. Thus, the viral concentration (pfu/mL) in the impinger was determined as:

$$\text{pfu/mL} = \frac{pfu}{10^{-n} \times V} \tag{9}$$

where *pfu* is the number of plaque forming units, *V* is the volume of diluted solution, and *n* is the dilution factor. The final viral concentration of impinger was determined by averaging viral concentrations in each dilution tube.

3.3.5 Viability of viruses on the filter

The viability of collected viral aerosol on the filter was measured following the same procedure used in the bacterial aerosol experiment. After 10 hrs of experiments the filter was transferred from the filter holder into a 200-mL beaker containing 40 mL sterile distilled water. After the designated vortexing time interval, two 1-mL samples, one for the original solution and the other for dilution, were withdrawn at each interval and subsequently assayed for plaques. The viability results of both filters were compared by using the survival fraction, the ratio of the viable count in the extract to the total collected on the filter for 10 hrs.

The effect on the extracted viral particles of iodine molecules released from the iodine-treated filter into the vortexing solution was considered by inoculating a known viral concentration into the vortexed solution passed through a clean iodine-treated filter to determine a correction factor for to the dissolved iodine. The exposure time of viral solution to each vortexed solution was 15 minutes at room temperature. Generally, iodine deactivates bacteriophages within a short contact time [34, 40]. The viable fraction was then adjusted by dividing the apparent relative fraction by the correction factor. The concentration of iodine in the vortexing solution was examined by a DPD colorimetric method adapted from *Standard Methods for the Examination of Water and Wastewater*, 4500-CIG. In a spectrophotometer (DR/4000 V Spectrophotometer, Hach), 10 mL of solution vortexed with the iodine-treated filter was analyzed at 530 nm. Iodine in the solution reacts with DPD (*N*, *N*-diethyl-*p*-phenylenediamine) to form a pink color. The color intensity is proportional to the total iodine concentration [59].

3.3.6 Shielding effect of viral aerosols

Viral suspensions at three concentrations, prepared by diluting portions of viral stock (0.1 mL, 0.5 mL, and 1 mL) with 150 mL of sterile deionized water, were delivered in turn as challenges to the iodine-treated filter for 30-min periods. Both entering and penetrating viral particles from the iodine-treated filter were collected in serially connected AGI-30 impingers and assayed after each 15 minutes of collection time. Each filter loaded with the viral particles was retrieved immediately after the bioaerosol challenge and subjected to the vortexing procedure. The survival fractions at each concentration were compared with one another to investigate the agglomeration effect on the survival of microorganisms.

4 ERROR AND UNCERTAINTY ANALYSIS [60–63]

Most experimental systems and measurements are subject to uncertainties. Therefore, errors and uncertainties need to be evaluated and reported properly to draw accurate conclusions. Evaluation of uncertainties is grouped into two categories: random/ indeterminate error and systematic/determinate error, depending on how their numerical values are estimated. Random error is evaluated by statistical analysis, while systematic error analysis is by means other than statistical analysis. All sources to be considered for this study are listed and investigated with respect to the type of influence they exert.

4.1 Random Error

4.1.1 Generation uncertainty

The characteristics of an ideal aerosol generator are a constant and reproducible output of stable aerosols with easily controlled size and concentration. The size distribution and concentration of bioaerosols depend on the characteristics of the generator and suspension in the nebulizer. A six-jet Collison nebulizer was employed to generate polydisperse bioaerosols. The suspension in the nebulizer consists of 0.1 mL microbial suspension and 150 mL sterilized deionized water. Since the suspension in the nebulizer is replaced by a new one every 2 hrs for the 10 hrs of experiment, the concentration of suspension varies amongst the 2-hr experimental sets. The variation of size distribution in each run of the experiment can be estimated by calculating the number fraction of each stage of the impactor. The values presented in Table 4.1 are averages and standard deviations for 30 replicate measurements.

Table 4.1. Fractional size distribution of bioaerosols collected on the cascade impactor

Stage No.	Size range (μm)	Number fraction	
		Average	Standard deviation
1	20–7.1	0.007	0.003
2	7.1–4.7	0.011	0.004
3	4.7–3.3	0.017	0.006
4	3.3–2.1	0.037	0.016
5	2.1–1.1	0.47	0.06
6	1.1–0.65	0.46	0.05

4.1.2 Scale uncertainty

Measurement errors are subdivided into random errors and unknown systematic errors. Systematic errors are induced by inexact adjustments and pre-settings of the environmental and boundary conditions. They remain constant during the entire measurement period as each is predetermined before the measurements begin. Therefore, there is a limit to treat measured data probabilistically for the calculation of systematic errors evaluated as calibration errors.

One source of random errors arises because the precision of measurement is limited by the instrument used to measure it. This inherent limitation is called the *scale error* or scale uncertainty of the instrument. In this filtration system, rotameters located in several positions to adjust flow rates can introduce scale uncertainty. A rotameter is composed of a metal float inside a conical glass tube. The float stays at a constant position at a constant flow rate. The smallest division on the scale of the rotameter is one digit. The position of the float can fluctuate due to temporary variations in the air supply during an experiment. As a worst-case estimation, the upper and lower two digits from the middle of the float are considered. The uncertainty of each rotameter in the system shown in Figure 4.1 is presented in Table 4.2. Flow rates were monitored during 30 experiments.

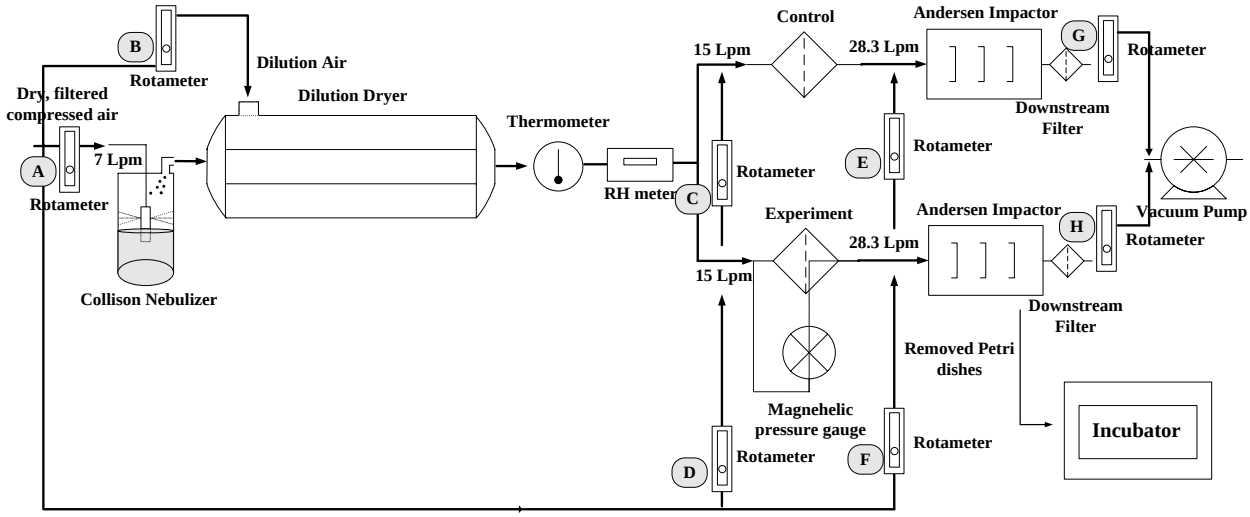


Figure 4.1. Rotameter position in bacterial aerosol experimental set-up

Table 4.2. Calculation of uncertainty in flow rate

Marks	Flow rate (Lpm)	Regression equation	R^2	Rotameter reading	Best estimate ± uncertainty (Lpm)
A	7	$y = 6.1270x + 8.1145$	0.9996	51 ± 2	6.9 ± 0.3
B	13	$y = 2.5597x – 3.2205$	0.9997	30 ± 2	13.0 ± 0.8
C	5	$y = 0.9549x – 0.3486$	0.9988	4.4 ± 0.2	5.0 ± 0.2
D	5	$y = 0.9592x – 0.3785$	0.9989	4.4 ± 0.2	5.0 ± 0.2
E	13.3	$y = 6.3016x + 6.4183$	0.9988	91 ± 2	13.4 ± 0.3
F	13.3	$y = 6.3188x + 8.2624$	0.9973	92 ± 2	13.3 ± 0.3
G	28.3	$y = 1.3193x – 3.2646$	0.9998	34 ± 2	28.2 ± 1.5
H	28.3	$y = 1.6309x + 0.4351$	0.9995	47 ± 2	28.5 ± 1.2

x: Flow rate, *y*: Rotameter reading

4.1.3 Experimenter error

Mistakes by an unskilled experimenter affect the experimental results. This can be overcome by repetition of practice and estimated from the reproducibility of experimental results. Table 4.3 lists the removal efficiency of each filter evaluated. As estimation of the uncertainty in the average of the measurements, standard error is also calculated. The statistical interpretation of standard error is that if the entire experiment is repeated with the same number of repetitions, the average value from the new experiment will be within one standard error of the average value from this experiment. The average removal efficiency of the second iodine-treated filter is 99.9994 %, which is within one standard error of that of the first iodine-treated filter: 99.9992 ± 0.0004 %.

Table 4.3. Removal efficiency (%) of evaluated filter

Filter media		Collection efficiency (%)		
		Average	Standard deviation	Standard error
Iodine-treated filter	1	99.9992	0.0008	0.0004
	2	99.9994	0.0008	0.0003
	3	99.9997	0.0008	0.0003
Untreated filter	1	99.9994	0.0008	0.0004
	2	99.9987	0.0018	0.0008
	3	99.9993	0.0009	0.0004

4.2 Systematic Error

4.2.1 Calibration uncertainty

Calibration error is the result of an improperly calibrated device. Careful design of calibration procedures will eliminate this kind of error. In this experiment, the rotameters used to adjust flow rate at various positions in the system are calibrated with a Gilibrator (#904008-H , Gilian Instrument Corp.) before conducting the 10-hr experiment. The calibration curve is obtained with at least three measurements at every designated rotameter reading with an R^2 value over 0.99.

4.2.2 Sampling error

When bioaerosols are collected on a sampling device, *e.g.*, an Andersen impactor for bacterial aerosols and AGI-30 impinger for viral aerosols, the phenomena of particle bounce, re-entrainment, and wall losses can alter the performance. The effect of particle bounce can be reduced by inserting a sticky agar plate into the impactor. However, the re-entrainment and wall losses are still present in the sampling process. Since such losses

happen simultaneously in both control and experiment sampling devices, those effects are offset by comparing both results.

Collected microorganisms must remain viable to be counted. However, many factors that affect the survival of microorganisms may occur during the sampling process. These include temperature, humidity, the presence of oxygen, and bacterial stress by shear forces. Therefore, collected microorganisms may not grow due to metabolic and structural injuries. Various counting methods such as staining methods, immunofluorescence microscopy or radioactive probes reduce this kind of uncertainties. In this experiment, only viable microorganisms are considered since the purpose of this study is the evaluation of iodine disinfection capacity which affects the viability of microorganisms by comparing experimental and control results. Microorganisms in both the experimental and control flow channel experience the same sampling stress resulting in the offset.

4.3 Combination of Uncertainties

Figure 4.2 presents a descriptive overview of the uncertainties in the experimental system.

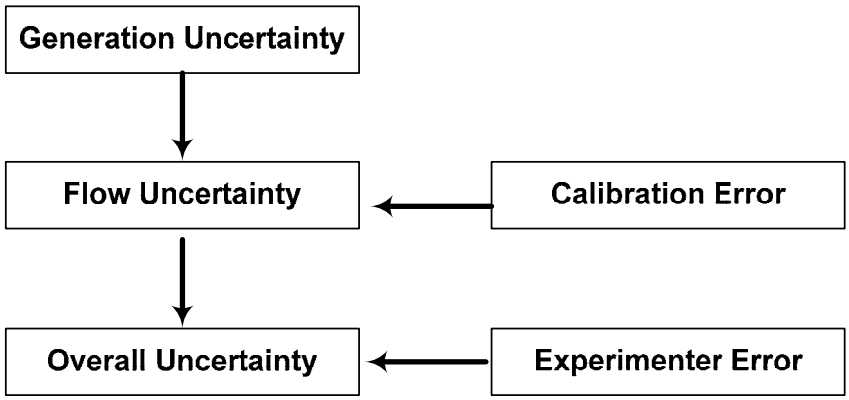


Figure 4.2. Flow of the uncertainties in experimental system

The experiment result, r, is a function of J variables X_i

$$r = r(X_1, X_2, \cdots, X_J) \tag{10}$$

Then the uncertainty in the results is given by

$$U_r = \left[\left(\frac{\partial r}{\partial X_1} U_{X_1} \right)^2 + \left(\frac{\partial r}{\partial X_2} U_{X_2} \right)^2 + \cdots + \left(\frac{\partial r}{\partial X_J} U_{X_J} \right)^2 \right]^{1/2} \tag{11}$$

where the Ux_i are the uncertainties in the measured variables X_i. The variables in this experimental system are generation and flow rates.

The total uncertainty of the generation was calculated by

$$U_{X_1} = \left[\left(\frac{Ux_{first}}{x_{first}} \right)^2 + \left(\frac{Ux_{second}}{x_{second}} \right)^2 + \cdots + \left(\frac{Ux_{sixth}}{x_{sixth}} \right)^2 \right]^{1/2} \quad (12)$$

where Ux_{first}, Ux_{second}, …. are the uncertainties of each stage of impactor presented by standard deviation, and x_{first}, x_{second},… is the number fraction of each stage.

Table 4.4. Total uncertainty of the generation process

Number Fraction	Standard Deviation	SD/NF	$(SD/NF)^2$
0.007	0.003	0.4	0.16
0.011	0.004	0.3	0.11
0.017	0.006	0.3	0.11
0.037	0.016	0.4	0.18
0.47	0.06	0.12	0.01
0.46	0.05	0.12	0.013
U_{X1}^2			0.6
U_{X1}			0.8

The total uncertainty of flow rate was also calculated by the same equation (12) used for generation.

Table 4.5. Total uncertainty of flow rates

Mark	Flow rate (Lpm)	Standard Deviation (Lpm)	SD/Flow rate	$(SD/Flow\ rate)^2$
A	6.9	0.3	0.04	0.0019
B	13	0.8	0.06	0.004
C	5	0.2	0.04	0.0016
D	5	0.2	0.04	0.0016
E	13.4	0.3	0.02	0.0005
F	13.3	0.3	0.02	0.0005
G	28.2	1.5	0.05	0.003
H	28.5	1.2	0.04	0.0018
U_{X2}^2				0.014
U_{X2}				0.12

To calculate the total uncertainty of the experimental system, it was assumed that the other variables are constant when each variable is considered to affect the final result and the mean of variance was adapted as the value of the derivative. The means of variance of result, generation, and flow rate are 1.16×10^{-7}, 0.6 and 1.07×10^{-3}, respectively. The calculated total uncertainty of this experimental system is as follows:

$$U_r = \left[\left(\frac{1.16\times10^{-7}}{0.6}\times 0.8\right)^2 + \left(\frac{1.16\times10^{-7}}{1.07\times10^{-3}}\times 0.12\right)^2\right]^{1/2} = 1.3\times10^{-5}$$

5 RESULTS

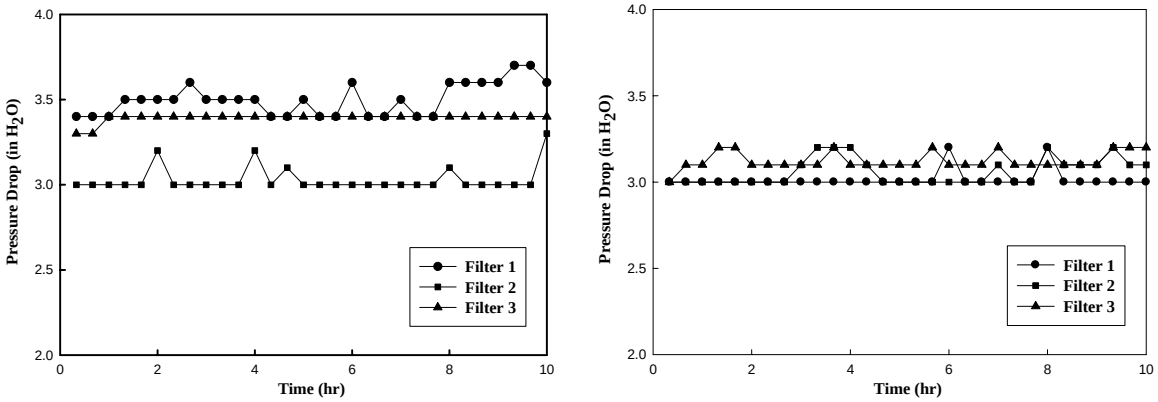
Experiments have been carried out for *B. subtilis* spores and MS2 bacteriophage using clean iodine-treated and untreated filters. Each experiment was carried out in triplicate.

5.1 Bacterial experiment

The bacterial experiment was conducted at low relative humidity (35 ± 5 %) and room temperature (23 ± 2 °C). Pressure drop across each filter was monitored using a Magnehelic gauge measuring 0–10 in H_2O and was recorded every 20 minutes. Raw data are presented in Appendix A.

5.1.1 Pressure drop

The initial pressure drop of the evaluated filters was 3.0~3.4 in H_2O. The measurements as a function of time are shown in Figure 5.1. All filters showed negligible variations in pressure drop throughout the 10-hr experiments.



(a) Iodine treated filter untreated filter

Figure 5.1. Pressure drop as a function of time for (a) iodine-treated and (b) untreated filters at low humidity (35 ± 5%) and room temperature (23 ± 2 °C).

To compare the pressure drop of the evaluated filter and a glass fiber HEPA filter (efficiency > 99.9997%), pressure drag, which is the measure of the filter's aerodynamic resistance to air flow, was calculated. The pressure drag of the evaluated filter was 0.009 inch H_2O/(in/min) whereas that of the glass fiber filter (Millipore AP 15) was 0.065 in H_2O/(in/min),. The lower pressure drag translates into less exertion to breathe through a respirator. The calculation is presented in Appendix D.

5.1.2 Physical removal efficiency

Figure 5.2 shows the size distribution of the entering bioaerosols collected by the impactor with no test filter. As shown, the majority of the entering bioaerosols were in the 0.65–2.1-μm range. Table 5.1 summarizes the results of each experiment at low humidity (35 ± 5 %) and room temperature (23 ± 2 ℃). Both the iodine-treated filter and the untreated filter displayed high collection efficiency (> 99.999%) during the 10-hr experiment. In most cases when the filter did not show complete collection efficiency, only one CFU penetration was detected downstream. It can also be seen that the efficiency did not deteriorate over time during the 10 hrs.

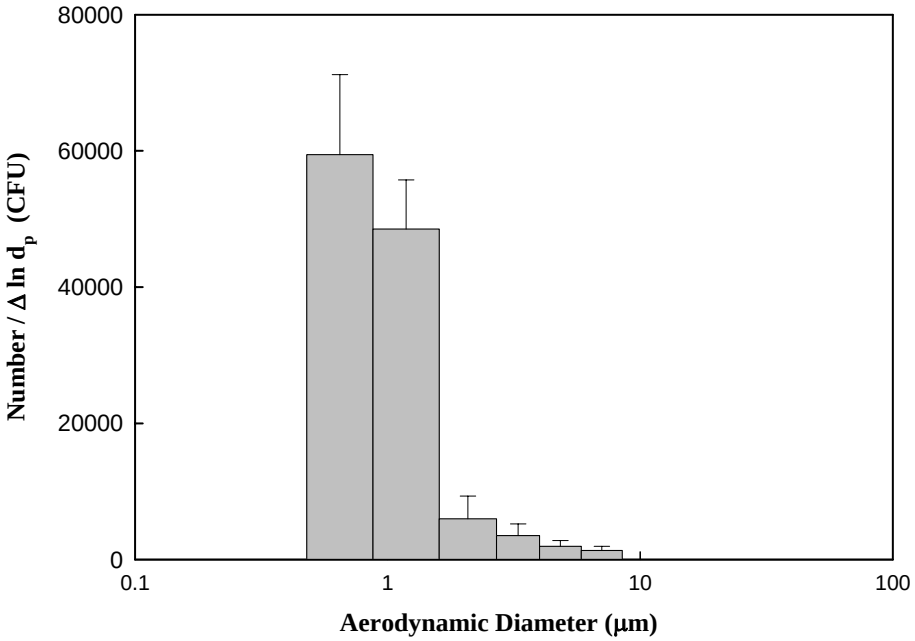


Figure 5.2. Particle size distribution of entering bioaerosols

Table 5.1. Removal efficiency (%) of filters for 10 hrs at low humidity (35 ± 5 %) and room temperature (23 ± 2 °C).

Filter Media		Filtration Time (hr)					
		2	4	6	8	10	Average ± S.D
Iodine-treated filter	No.1	100	99.9989	99.9981	99.9988	100	99.9994 ± 0.0008
	No.2	99.9988	100	99.9985	100	100	
	No.3	100	99.9983	100	100	100	
Untreated filter	No.1	100	100	99.9984	100	99.9985	99.9991 ± 0.0012
	No.2	100	100	99.9968	100	99.9965	
	No.3	100	100	99.9983	100	99.9984	

5.1.3 Survival fraction

Table 5.2 shows the viable counts of microorganisms extracted from both filters at each vortexing time. A slightly higher number of extracted microorganisms were enumerated from the untreated filter than from iodine-treated filter.

Table 5.2. Viable microorganisms (CFU) extracted from both filters

Filter Media		Vortex Time (min)				
		1	2	3	5	10
Iodine-treated filter	No.1	200	219	918	204	120
	No.2	260	114	108	221	64
	No.3	240	228	108	136	224
Untreated filter	No.1	400	836	792	561	944
	No.2	320	361	504	442	320
	No.3	580	646	882	1054	2864

The viability of the collected microorganisms is presented in Table 5.3 as a survival fraction, calculated by dividing the extracted microorganisms with the total microorganisms (C_E/C_C), C_E: Extracted microorganisms, C_C: Microorganisms collected on the filter during 10 hrs). The average survival fraction was calculated by considering all vortexing times. The survival fraction of the iodine-treated filter was lower than that of the untreated filter, although both survival fractions were low.

Table 5.3. Survival fractions of microorganisms on both filters

Filter Media		Average ± S.D	Average ± S.D
Iodine-treated filter	No.1	7E-04 ± 7E-04	5.9E-04 ± 1.3E-04
	No.2	4E-04 ± 2E-04	
	No.3	6E-04 ± 2E-04	
Untreated filter	No.1	2.1E-03 ± 6E-04	2.5E-03 ± 1.4E-03
	No.2	1.4E-03 ± 3E-04	
	No.3	4E-03 ± 3E-03	

The experimental result on the effect of free iodine residual in the extract solution is presented in Table 5.4, where the average of three filters is displayed. As shown, iodine extracted from the iodine-treated filter during vortexing can affect the viability of microorganisms in the solution. Accordingly, the survival fraction of microorganisms on the iodine-treated filter reported in Table 5.4 needs to be corrected. However, it is observed that the amount of iodine extracted does not increase as vortexing time increases. The method of correction is described below.

The effect of vortexing on the infectivity of microorganisms was also a concern. A spore suspension of known composition of was vortexed for each designated time and remaining viability was examined. The relative fraction obtained by dividing the number

Table 5.4. Relative fraction of microorganisms in the vortexed solution and the control

Vortexing Time	Iodine-treated filter	Untreated filter
	Average ± S.D	Average ± S.D
1	0.83 ± 0.05	0.99 ± 0.06
2	0.86 ± 0.05	0.98 ± 0.04
3	0.86 ± 0.03	1.03 ± 0.08
5	0.87 ± 0.05	0.99 ± 0.04
10	0.86 ± 0.02	1.04 ± 0.07
Total	0.856 ± 0.014	1.01 ± 0.03

of viable microorganisms at each vortexing time by those at zero vortexing time is presented in Table 5.5. It is observed that the vortexing effect on the viability of bacterial spores for 10 mins of vortexing time is negligible. The corrected survival fraction considering the effect of free iodine residuals is presented in Table 5.6.

Table 5.5. Relative fraction of viable microorganisms at each vortexing time

Experiment run	Vortex Time (min)				
	1	2	3	5	10
1	1.02	0.93	0.97	0.96	0.96
2	1.2	1.3	1.3	1.2	0.9
3	1.1	0.9	0.97	0.98	0.8

Table 5.6. Corrected survival fraction of microorganisms on the iodine-treated filter

Filter Media		Average ± S.D	Average ± S.D
Iodine-treated filter	No.1	8E-04 ± 8E-04	6.9E-04 ± 1.6E-04
	No.2	5E-04 ± 3E-04	
	No.3	7E-04 ± 2E-04	

5.2 Viral experiment

Viral experiments were conducted at low relative humidity (35 ± 5 %) and room temperature (23 ± 2℃). It should be emphasized again that filter media different from those used in bacterial experiment were tested. Pressure drop across each filter was monitored with a Magnehelic gauge measuring 0–10 in H_2O and recorded every 30 minutes. The raw data are available in Appendix B.

5.2.1 Pressure drop

The initial pressure drop of tested filters was 0.2 inch H_2O; variation in the pressure drop during 10 hrs of experiment was almost negligible.

Pressure drag was calculated to compare the pressure drop of the tested filter with those of filters evaluated in bacterial experiments and with the glass fiber filter. Pressure drag of the filters in the viral experiment was 0.0006 in H_2O/(in/min); of the glass fiber filter (Millipore AP 15), 0.065 in H_2O/(in/min); and of the filter used in the bacterial spore experiment, 0.009 in H_2O/(in/min). The filter evaluated in the viral experiment presented a much lower pressure drag than those used in the bacterial spore experiment. The calculation is available in Appendix D.

5.2.2 Physical removal efficiency

Physical removal efficiency of the filters tested is presented in Table 5.7. The result of each 2-hr filtration session is the average of experimental results of four consecutive 30-min runs because the liquid in impingers was replaced by a new charge and assayed every 30 min. The removal efficiency apparently is lower than the filter used in bacterial spore experiments. It is not clear whether the difference is due to different particle size or the different filter medium. Unfortunately, there was not enough of either filter medium to perform all experiments for a fair comparison.

Table 5.7. Removal efficiency (%) of both filters for 10 hrs at low humidity (35 ± 5 %) and room temperature (23 ± 2 °C)

Filter Medium		Filtration Time (hr)					
		2	4	6	8	10	Total
Iodine-treated filter	No.1	93 ± 3	96.9 ± 1.4	93 ± 3	94 ± 4	93 ± 3	94 ± 3
	No.2	93.2 ± 0.6	95.9 ± 1.5	94 ± 2	95 ± 3	95 ± 3	
	No.3	91.1 ± 1.9	93 ± 3	94.1 ± 1.7	92 ± 3	90.5 ± 0.8	
Untreated filter	No.1	90.3 ± 1.6	94.6 ± 1.3	92.1 ± 1.1	92.7 ± 1.3	92.3 ± 0.3	92 ± 2
	No.2	91.0 ± 1.9	90 ± 4	90.9 ± 1.8	90.4 ± 1.4	91.6 ± 1.5	
	No.3	94.8 ± 1.7	91 ± 3	90.4 ± 1.2	92.2 ± 0.6	92.6 ± 0.9	

5.2.3 Survival fraction

Table 5.8 presents the viable counts of microorganisms extracted from the tested filter at each vortexing time. The results did not show a specific trend of increasing or decreasing viability of extracted microorganisms.

Survival fraction (C_E/C_C), C_E: Extracted microorganisms, C_C: Microorganisms collected on the filter during 10 hrs) was calculated to determine the infectivity of collected microorganisms. Table 5.9 summarizes the survival fractions atf all vortexing times. As shown, the survival fraction of the iodine-treated filter is 10 times lower than that of the untreated filter.

Table 5.8. Viable microorganisms (PFU) extracted from both filters

Filter Media		Vortex Time (min)				
		1	2	3	5	10
Iodine-treated filter	No.1	4400	1194	22176	15760	45472
	No.2	26200	24278	28116	27186	23128
	No.3	23600	18308	27324	25610	33320
Untreated filter	No.1	93200	238800	215622	100864	195804
	No.2	111800	71242	545094	63434	407876
	No.3	73200	88356	84744	105168	92120

Table 5.9. Survival fraction of microorganisms on both filters

Filter Media		Average ± S.D	Average ± S.D
Iodine-treated filter	No.1	$2 \times 10^{-03} \pm 2 \times 10^{-03}$	$3.7 \times 10^{-03} \pm 1.3 \times 10^{-03}$
	No.2	$4.8 \times 10^{-03} \pm 4 \times 10^{-04}$	
	No.3	$4.0 \times 10^{-03} \pm 9 \times 10^{-04}$	
Untreated filter	No.1	$3.0 \times 10^{-02} \pm 1.2 \times 10^{-02}$	$4 \times 10^{-02} \pm 3 \times 10^{-02}$
	No.2	$7 \times 10^{-02} \pm 7 \times 10^{-02}$	
	No.3	$2.2 \times 10^{-02} \pm 3 \times 10^{-03}$	

The same procedure used in the bacterial experiment for assessing the effect of vortexing alone was followed. The results presented as relative fraction are shown in Table 5.10. The value suggests that 10 mins of vortexing did not affect the infectivity of virus. Hence, there is no need to correct the survival fraction for the vortexing effect.

Table 5.10. Relative fraction of infectivity of microorganisms in each vortexing time

Experiment run	Vortex Time (min)				
	1	2	3	5	10
1	1.3	1.1	1.0	1.3	1.0
2	1.0	0.9	0.9	0.9	1.0
3	1.0	1.3	1.1	1.4	1.0

As in the bacterial experiment, effects of vortexing and of free iodine on infectivity of microorganisms in the solutions are concerns. The same experimental procedures used in the bacterial spore experiments for vortexing with clean iodine-treated filters were followed. Three virus concentrations, adjusted by dilution, were used. One mL of the solution vortexed with clean iodine-treated filter was inoculated with 0.1 mL of virus suspension of known concentration. After 15 min of exposure time, this mixed solution was assayed to determine the amount of surviving virus. As a control, 0.1 mL of stock virus suspension was mixed with 1 mL of sterilized deionized water. The relative fraction, shown in Table 5.11, was calculated by dividing the plaque count at each vortexing time with that of the control.

Table 5.11. Relative fraction of microorganisms in the vortexed solution

Number of Plaques Measured in the Control	Vortexing time (min)				
	1	2	3	5	10
36	0.03	0.14	0.08	0.19	0.06
66	0.05	0.2	0.5	0.05	0.2
163	0.02	0.12	0.2	0.06	0.05
294	0.4	0.2	0.17	0.5	0.3
441	0.12	0.15	0.18	0.17	0.14

Statistical analysis (ANOVA) was performed against two factors: virus concentration and vortexing time. Results of the analysis are presented in Appendix E. Both *p*-values—for concentration (0.018) and for vortexing time (0.8)—are higher than 0.005, indicating that neither concentration nor vortexing time affects the experiment results. The average value of the relative fraction, 0.17, was used to correct the survival fraction of microorganisms extracted from the iodine-treated filter. The adjusted values are presented in Table 5.12. As shown, the mean adjusted values are still lower than those of the untreated filter (~50%).

Table 5.12. Corrected survival fraction of microorganisms on iodine-treated filters

Filter Medium		Average ± S.D	Average ± S.D
Iodine-treated filter	No.1	$1.3 \times 10^{-02} \pm 1.3 \times 10^{-02}$	$2.2 \times 10^{-02} \pm 8 \times 10^{-03}$
	No.2	$2.8 \times 10^{-02} \pm 2 \times 10^{-03}$	
	No. 3	$2.4 \times 10^{-02} \pm 5 \times 10^{-03}$	

5.2.4 Iodine concentration in the vortexing solution

The iodine concentration in the vortexing solution was analyzed. The results are presented in Table 5.13. It can be seen that some iodine is released from the iodine-treated filter before the start of vortexing (designated as “0” vortexing time). No further increase of iodine extraction from the filter by increasing vortexing time was observed. The average iodine concentration at all vortexing times is around 1.0 mg/L of I_2.

Table 5.1. Iodine concentration (mg/L) from iodine-treated medium in the vortexing solution

Filter Medium		Vortex Time (min)			
		0	1	5	10
	No.1	0.74	1.02	0.82	0.94
	No.2	0.52	0.96	0.86	0.92

5.2.5 Shielding effect of agglomerated virus

The removal efficiency of the iodine-treated filter is presented in Table 5.14 for three viral concentrations Duplicate experiments were conducted.

Table 5.14. Removal efficiency (%) of iodine-treated filter for three concentrations of MS2

Filter Media		Viral stock (mL)		
		0.1	0.5	1
Iodine-treated filter	No.1	96.56	93.45	99.82
	No.2	97.64	98.66	98.45

Table 5.15 shows the relative fraction of viral particles extracted from the iodine-treated filter challenged with different feed concentrations. Based on results in the control (without filter) and experiment (with filter), the number of viral particles collected on the filter was calculated. A higher number of viral particles collected on the filter corresponds to a higher degree of viral agglomeration, as it is hypothesized that a higher degree of viral agglomeration results in more-effective shielding.

Table 5.15. Relative fraction of microorganisms in different agglomeration

Viral particles collected on the filter (PFU)	Vortex Time (min)			
	1	5	10	Average
$1.5 \times 10^{+07}$	0.04	0.13	0.17	0.11
$1.8 \times 10^{+07}$	0.10	0.11	0.23	0.15
$2.1 \times 10^{+07}$	0.17	0.28	0.30	0.25
$1.0 \times 10^{+11}$	0.4	0.4	0.6	0.5
$1.4 \times 10^{+15}$	0.16	0.12	0.13	0.14
$3.5 \times 10^{+15}$	0.02	0.2	0.2	0.15

As shown in Table 5.14, there appears to be no significant difference in the survival fraction between low and high degrees of agglomeration. There are several possible inter-pretations for the observed phenomena. The degree of agglomeration studied in this experiment may not be enough to provide effective shielding for viral particles on the inside. Whether the reaction time of iodine with viral particles to deactivate the infectivity of virus is enough is not known. It is also possible that viral agglomerates dislodged from the filter disagglomerated in the solution and voided the shielding effect. The results obtained in this study are preliminary in nature and additional study is needed to clarify the effects and reasons.

6 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Removal efficiencies of the iodine-treated and the untreated filter during the 10-hr experiment with bacterial spore aerosols were $99.9994 \pm 0.0008\%$ and $99.9991 \pm 0.0012\%$, respectively. Pressure drop of the filters evaluated was 3.0~3.4 in H_2O initially, and the variation in pressure drop during 10 hrs was negligible. The pressure drag of the filter (0.009 inch H_2O/(in/min)) tested was much lower than that of the glass fiber HEPA filter (0.065 inch H_2O/(in/min)) tested. Viability of microorganisms collected on the filter was investigated by vortexing the filter after the 10-hr bacterial spore experiment and was presented as the survival fraction. Because free iodine extracted from the filter during vortexing can affect the viability of microorganisms in the solution, the survival fraction was adjusted to exclude this effect. The corrected values of the iodine-treated and untreated filter were $6.9 \times 10^{-4} \pm 1.6 \times 10^{-4}$ and $2.5 \times 10^{-3} \pm 1.4 \times 10^{-3}$, respectively.

For the viral experiment, AFRL supplied new filter media samples different from those used in the bacterial spore experiment. The removal efficiencies of the iodine-treated and untreated filter for viral aerosols were 94 ± 3 % and 92 ± 2 %, respectively. Initial pressure drop was very low (0.2 in H_2O) and remained almost constant during the 10-hr experiment. The pressure drag was 0.0006 inch H_2O/(in/min), much lower than that of filters evaluated in the bacterial spore experiment. The free iodine effect was investigated and the average value (0.17) was used for correction. The survival fractions of viral aerosols collected on the iodine-treated and untreated filter were $2.2 \times 10^{-2} \pm 8 \times 10^{-3}$ and $4 \times 10^{-2} \pm 3 \times 10^{-2}$, respectively.

No significant shielding effect was observed with the various degrees of agglomeration of viral particles used in this research. The relative survival fractions of both low and high degrees of agglomeration were similar.

6.2 Conclusions

Several conclusions can be drawn from the experimental results:

(1) Iodine-treated filters presented high removal efficiency of bacterial spores with low pressure drop compared to the HEPA filter. Meanwhile, the untreated filter also exhibited similar performance.

(2) Compared with the performance of untreated filters, the iodine-treated filter causes measurable inactivation of collected bacterial spores.

(3) The filter media used for virus experiments showed lower removal efficiency than that of bacterial spore experiments. This is presumably due mainly to the size of the test aerosol but the influence of the difference in the media remains an open question. Both iodine-treated and untreated filters showed similar removal efficiency in the virus experiment.

(4) Based on the survival fraction, the iodine-treated filter showed a higher deactivation capacity for MS2 bacteriophage than untreated filter. However, they are not statistically different due to the large variation.

(5) no significant shielding effect of agglomerated particles was observed in the concentration range we investigated.

6.3 Recommendations

Further research is recommended to establish the effectiveness of reactive media as a component of biocidal filters for versatile applications in various circumstances. In this study, the effectiveness of iodine-treated filters was evaluated at low humidity and room temperature as environmental factors. It is suspected, however, that the disinfection efficacy of iodine-treated filters will increase at high humidity and temperature due to the dissolution and sublimation of iodine.

In the operation of filtration systems and respirators, the filter collects all varieties of aerosols—*e.g.*, mineral dust particles and particles generated from combustion sources—in addition to bioaerosols. The presence of these particles may hinder the exertion of biocidal effect by interaction with active sites of the filter that would otherwise react with microbes. Furthermore, these substances could serve as nutrients for the growth of collected microorganisms, resulting in the inhalation of reentrained bioaerosols. Accordingly, the disinfection capacity of iodine-treated filter media under conditions that may be expected to diminish the effectiveness of iodine should be investigated.

For future experiments, certain considerations in the experimental methodology are recommended. The adoption of thiosulfate solution as the vortexing solution instead of sterile deionized water used in this study may exclude the effect of free iodine extracted from the filter by reducing the iodine species to iodide. Moreover, accurate characterization of agglomerated bioaerosols will be helpful to clarify the relationship between the shielding effect and agglomeration of biological particles. The method used in the experiment to measure the shielding effect of bioaerosols could only qualitatively provide the degree of agglomeration by changing the viral suspension concentration in the reservoir, so it could not be used to accurately determine the size of agglomerates.

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Appendix A. Bacterial Experiment Raw Data

Iodine-treated filter 1					
Experiment 1.		Temperature : 23 ℃, Barometric Pressure : 30.23 in. Hg			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.4	34	0	1	1248
40	3.4	32	0	2	1812
60	3.4	32	0	3	3096
80	3.5	32	0	4	5712
100	3.5	33	0	5	28068
120	3.5	32	0	6	58248
Experiment 2.		Temperature : 24.5 ℃, Barometric Pressure : 29.94 in. Hg			
20	3.5	36	0	1	888
40	3.6	32	0	2	1860
60	3.5	32	1	3	2724
80	3.5	33	0	4	4920
100	3.5	33	0	5	35616
120	3.5	33	0	6	48552
Experiment 3.		Temperature : 23 ℃, Barometric Pressure : 29.94 in. Hg			
20	3.4	38	0	1	960
40	3.4	33	1	2	1572
60	3.5	32	1	3	2904
80	3.4	33	0	4	11628
100	3.4	33	0	5	44580
120	3.6	32	0	6	44304
Experiment 4.		Temperature : 23 ℃, Barometric Pressure : 29.92 in. Hg			
20	3.4	38	1	1	792
40	3.4	33	0	2	936
60	3.5	32	0	3	1884
80	3.4	33	0	4	3024
100	3.4	33	0	5	44580
120	3.6	32	0	6	35616
Experiment 5		Temperature : 23 ℃, Barometric Pressure : 29.92 in. Hg			
20	3.6	35	0	1	408
40	3.6	34	0	2	936
60	3.6	32	0	3	1428
80	3.7	32	0	4	3228
100	3.7	33	0	5	42960
120	3.6	34	0	6	29520

* CFU is the number of microorganism normalized to 120 minutes.

Iodine-treated filter 2					
Experiment 1.		Temperature : 23 ℃, Barometric Pressure : 30.01 in. Hg			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.0	34	0	1	552
40	3.0	33	1	2	612
60	3.0	33	0	3	1212
80	3.0	33	0	4	2388
100	3.0	33	0	5	31344
120	3.2	33	0	6	44016
Experiment 2.		Temperature : 23 ℃, Barometric Pressure : 30.01 in. Hg			
20	3.0	40	0	1	792
40	3.0	38	0	2	948
60	3.0	34	0	3	1644
80	3.0	34	0	4	2616
100	3.0	33	0	5	33372
120	3.2	33	0	6	25884
Experiment 3.		Temperature : 23.5 ℃, Barometric Pressure : 29.94 in. Hg			
20	3.0	38	0	1	360
40	3.1	36	0	2	432
60	3.0	33	0	3	1008
80	3.0	32	1	4	2064
100	3.0	32	0	5	30192
120	3.0	32	0	6	31200
Experiment 4.		Temperature : 23.5 ℃, Barometric Pressure : 29.94 in. Hg			
20	3.0	39	0	1	264
40	3.0	38	0	2	564
60	3.0	35	0	3	780
80	3.0	33	0	4	2088
100	3.0	33	0	5	30144
120	3.1	33	0	6	25944
Experiment 5		Temperature : 23.5 ℃, Barometric Pressure : 29.94 in. Hg			
20	3.0	39	0	1	516
40	3.0	37	0	2	900
60	3.0	34	0	3	1200
80	3.0	33	0	4	2484
100	3.0	32	0	5	34548
120	3.3	32	0	6	31752

Iodine-treated filter 3					
Experiment 1.		Temperature : 23.5 ℃, Barometric Pressure : 29.70 in. Hg			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.3	39	0	1	204
40	3.3	34	0	2	420
60	3.4	33	0	3	600
80	3.4	32	0	4	1380
100	3.4	32	0	5	25536
120	3.4	32	0	6	21192
Experiment 2.		Temperature : 23 ℃, Barometric Pressure : 29.63 in. Hg			
20	3.4	40	0	1	492
40	3.4	35	0	2	552
60	3.4	35	0	3	1080
80	3.4	34	0	4	2028
100	3.4	33	0	5	27624
120	3.4	32	1	6	26100
Experiment 3.		Temperature : 23 ℃, Barometric Pressure : 29.63 in. Hg			
20	3.4	40	0	1	168
40	3.4	40	0	2	348
60	3.4	37	0	3	600
80	3.4	36	0	4	1488
100	3.4	35	0	5	25620
120	3.4	32	0	6	22680
Experiment 4.		Temperature : 23 ℃, Barometric Pressure : 29.86 in. Hg			
20	3.4	39	0	1	564
40	3.4	36	0	2	1104
60	3.4	34	0	3	1800
80	3.4	34	0	4	3408
100	3.4	33	0	5	30468
120	3.4	35	0	6	40368
Experiment 5		Temperature : 23 ℃, Barometric Pressure : 29.83 in. Hg			
20	3.4	40	0	1	360
40	3.4	40	0	2	660
60	3.4	37	0	3	1116
80	3.4	36	0	4	2400
100	3.4	35	0	5	31860
120	3.4	35	0	6	35436

Untreated filter 1					
Experiment 1.		Temperature : 24.5 ℃, Barometric Pressure : 29.92 in. Hg			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.0	38	0	1	720
40	3.0	36	0	2	828
60	3.0	34	0	3	1260
80	3.0	33	0	4	2472
100	3.0	33	0	5	36264
120	3.0	33	0	6	33324
Experiment 2.		Temperature : 24.5 ℃, Barometric Pressure : 29.92 in. Hg			
20	3.0	39	0	1	684
40	3.0	37	0	2	1008
60	3.0	34	0	3	1632
80	3.0	33	0	4	3060
100	3.0	33	0	5	42960
120	3.0	32	0	6	37884
Experiment 3.		Temperature : 24.5 ℃, Barometric Pressure : 29.95 in. Hg			
20	3.0	39	0	1	660
40	3.0	34	1	2	912
60	3.0	33	0	3	1380
80	3.0	33	0	4	2700
100	3.0	33	0	5	32292
120	3.2	33	0	6	26484
Experiment 4.		Temperature : 24.5 ℃, Barometric Pressure : 30.04 in. Hg			
20	3.0	39	0	1	252
40	3.0	35	0	2	396
60	3.0	35	0	3	492
80	3.0	34	0	4	1560
100	3.0	34	0	5	24672
120	3.2	33	0	6	20604
Experiment 5		Temperature : 24.5 ℃, Barometric Pressure : 30.06 in. Hg			
20	3.0	40	0	1	408
40	3.0	38	0	2	936
60	3.0	36	1	3	1428
80	3.0	34	0	4	3228
100	3.0	33	0	5	34092
120	3.0	33	0	6	27060

Untreated filter 2					
Experiment 1.		Temperature : 23 ℃, Barometric Pressure : 29.86 in. Hg			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.0	40	0	1	288
40	3.0	40	0	2	552
60	3.0	38	0	3	612
80	3.0	34	0	4	1368
100	3.0	34	0	5	28644
120	3.0	33	0	6	20724
Experiment 2.		Temperature : 23 ℃, Barometric Pressure : 29.86 in. Hg			
20	3.0	35	0	1	120
40	3.0	35	0	2	252
60	3.1	35	0	3	648
80	3.2	34	0	4	960
100	3.2	33	0	5	19788
120	3.2	33	0	6	20484
Experiment 3.		Temperature : 23 ℃, Barometric Pressure : 30.08 in. Hg			
20	3.1	38	1	1	480
40	3.0	33	1	2	816
60	3.0	33	0	3	1260
80	3.0	33	0	4	2412
100	3.0	33	0	5	29232
120	3.0	33	0	6	28572
Experiment 4.		Temperature : 23 ℃, Barometric Pressure : 30.23 in. Hg			
20	3.0	40	0	1	168
40	3.0	40	0	2	672
60	3.1	38	0	3	1044
80	3.0	36	0	4	2088
100	3.0	33	0	5	30600
120	3.2	33	0	6	35472
Experiment 5		Temperature : 24 ℃, Barometric Pressure : 29.91 in. Hg			
20	3.1	40	0	1	384
40	3.1	36	0	2	768
60	3.1	34	0	3	1116
80	3.2	33	2	4	2148
100	3.1	34	0	5	29472
120	3.1	34	0	6	22608

Untreated filter 3					
Experiment 1.		Temperature : 23 ℃, Barometric Pressure : 29.92 in. Hg			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.0	53.0	0	1	300
40	3.1	53	0	2	456
60	3.1	52	0	3	636
80	3.2	49	0	4	1296
100	3.2	48	0	5	27684
120	3.1	46	0	6	36816
Experiment 2.		Temperature : 22.5 ℃, Barometric Pressure : 29.92 in. Hg			
20	3.1	40	0	1	144
40	3.1	39	0	2	372
60	3.1	39	0	3	780
80	3.1	39	0	4	1392
100	3.2	38	0	5	28068
120	3.1	38	0	6	29412
Experiment 3.		Temperature : 23.5 ℃, Barometric Pressure : 29.89 in. Hg			
20	3.1	40	0	1	276
40	3.1	40	1	2	552
60	3.1	37	0	3	588
80	3.1	36	0	4	2112
100	3.2	35	0	5	25200
120	3.1	36	0	6	30348
Experiment 4.		Temperature : 23.5 ℃, Barometric Pressure : 29.89 in. Hg			
20	3.1	37	0	1	276
40	3.1	37	0	2	324
60	3.2	35	0	3	480
80	3.1	35	0	4	1284
100	3.1	36	0	5	24924
120	3.1	36	0	6	21528
Experiment 5		Temperature : 24 ℃, Barometric Pressure : 29.91 in. Hg			
20	3.1	40	0	1	456
40	3.1	38	1	2	444
60	3.1	36	0	3	744
80	3.2	35	0	4	2136
100	3.2	36	0	5	30924
120	3.2	36	0	6	25944

Appendix B. Viral Experiment Raw Data

Iodine-treated filter 1							
Experiment 1.		Temperature : 23 ℃, Barometric Pressure : 29.98 in. Hg					
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)		Control (PFU/mL)		Removal Eff. (%)
			Impinger-1	Impinger-2	Impinger-1	Impinger-2	
30	0.2	38	1195	1410	23300	25050	94.61
60	0.3	39	1890	300	9500	16450	91.56
90	0.3	39	750	1250	15050	37500	96.19
120	0.2	38	685	530	10850	13100	94.93
Experiment 2.		Temperature : 22 ℃, Barometric Pressure : 30.01in. Hg					
30	0.2	40	685	530	10850	13100	94.93
60	0.3	38	200	410	14300	7000	97.14
90	0.2	38	275	295	14700	6700	97.34
120	0.2	37	540	455	28000	30650	98.30
Experiment 3.		Temperature : 23.5 ℃, Barometric Pressure : 30.16in. Hg					
30	0.2	40	300	325	1350	6000	91.50
60	0.2	39	350	1805	7000	13800	89.64
90	0.2	39	165	290	4200	5950	95.52
120	0.2	38	1100	215	800	30500	95.80
Experiment 4.		Temperature : 23 ℃, Barometric Pressure : 30.05in. Hg					
30	0.2	39	115	165	4600	5300	97.17
60	0.2	38	200	210	400	4500	91.63
90	0.2	39	105	325	900	3350	89.88
120	0.3	37	70	130	800	5850	96.99
Experiment 5.		Temperature : 23 ℃, Barometric Pressure : 30.05in. Hg					
30	0.2	39	185	120	1500	1500	89.83
60	0.2	38	100	20	400	1000	91.43
90	0.2	38	70	30	300	1350	93.94
120	0.2	37	200	300	2600	8950	95.67

Iodine-treated filter 2							
Experiment 1.		Temperature : 23 ℃, Barometric Pressure : 29.98 in. Hg					
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)		Control (PFU/mL)		Removal Eff. (%)
			Impinger-1	Impinger-2	Impinger-1	Impinger-2	
30	0.2	40	700	390	3550	12000	92.99
60	0.2	39	240	165	3500	1850	92.43
90	0.2	39	195	600	5150	7500	93.72
120	0.2	39	225	275	4750	3250	93.75
Experiment 2.		Temperature : 23 ℃, Barometric Pressure : 29.98in. Hg					
30	0.2	40	700	390	9050	13000	95.06
60	0.2	39	200	600	14300	7000	96.24
90	0.2	39	215	250	6900	14200	97.80
120	0.2	40	410	505	7300	8800	94.32
Experiment 3.		Temperature : 23 ℃, Barometric Pressure : 30.01in. Hg					
30	0.2	40	205	500	4700	4350	92.21
60	0.2	38	330	745	19600	27450	97.72
90	0.3	38	900	760	9600	19300	94.26
120	0.3	37	800	255	5750	10050	93.32
Experiment 4.		Temperature : 22 ℃, Barometric Pressure : 29.74 in. Hg					
30	0.2	39	105	20	5450	2150	98.36
60	0.2	39	60	10	1300	1000	96.96
90	0.2	38	255	240	4700	3350	93.85
120	0.2	38	480	240	2700	6750	92.38
Experiment 5.		Temperature : 23 ℃, Barometric Pressure : 30.22in. Hg					
30	0.2	40	420	680	6750	5050	90.68
60	0.2	39	160	230	3650	4350	95.13
90	0.2	40	150	295	1700	3400	91.27
120	0.2	38	170	150	2550	5850	96.19

Iodine-treated filter 3							
Experiment 1.		Temperature : 22 ℃, Barometric Pressure : 30.15 in. Hg					
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)		Control (PFU/mL)		Removal Eff. (%)
			Impinger-1	Impinger-2	Impinger-1	Impinger-2	
30	0.2	40	630	860	4700	9750	89.69
60	0.2	39	2545	3225	10300	82600	93.79
90	0.2	39	1305	645	4500	14350	89.66
120	0.2	39	1165	650	12800	7600	91.10
Experiment 2.		Temperature : 23 ℃, Barometric Pressure : 30.05in. Hg					
30	0.2	40	425	355	3100	6000	91.43
60	0.2	39	155	110	3300	2950	95.76
90	0.2	39	50	285	2150	1600	91.07
120	0.2	40	85	95	1500	2500	95.50
Experiment 3.		Temperature : 22 ℃, Barometric Pressure : 30.10in. Hg					
30	0.2	40	85	70	1750	950	94.26
60	0.2	40	115	240	6250	3000	96.16
90	0.2	39	140	105	1900	2050	93.80
120	0.2	40	175	90	800	2550	92.09
Experiment 4.		Temperature : 22 ℃, Barometric Pressure : 29.87 in. Hg					
30	0.2	40	650	745	5050	8200	89.47
60	0.2	40	345	620	12300	7850	95.21
90	0.2	40	220	175	1250	2400	89.18
120	0.2	40	315	480	3900	7900	93.26
Experiment 5.		Temperature : 22 ℃, Barometric Pressure : 29.87 in. Hg					
30	0.2	40	945	1320	11500	12650	90.62
60	0.2	40	870	2155	14050	14800	89.51
90	0.2	39	640	1850	12100	17300	91.53
120	0.2	40	780	1725	6100	19450	90.20

Untreated filter 1							
Experiment 1.		Temperature : 23 ℃, Barometric Pressure : 30.27 in. Hg					
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)		Control (PFU/mL)		Removal Eff. (%)
			Impinger-1	Impinger-2	Impinger-1	Impinger-2	
30	0.2	40	1255	1065	11400	10300	89.31
60	0.2	40	1210	905	12950	12650	91.74
90	0.2	39	1295	1110	9700	11500	88.66
120	0.2	38	1380	990	15450	12900	91.64
Experiment 2.		Temperature : 23 ℃, Barometric Pressure : 29.87in. Hg					
30	0.2	40	170	175	3200	4200	95.34
60	0.2	39	125	425	6150	5500	95.28
90	0.2	38	50	520	4700	3100	92.69
120	0.2	38	205	145	4800	2500	95.21
Experiment 3.		Temperature : 23.5 ℃, Barometric Pressure : 29.84in. Hg					
30	0.2	40	1845	1635	7900	39950	92.73
60	0.2	39	1325	1495	16900	13050	90.58
90	0.2	38	580	1270	10450	15750	92.94
120	0.2	38	1340	970	10300	18600	92.01
Experiment 4.		Temperature : 22 ℃, Barometric Pressure : 30.00 in. Hg					
30	0.2	39	235	305	2100	3900	91.00
60	0.2	38	175	95	1450	2100	92.39
90	0.2	38	70	115	1050	2000	93.93
120	0.2	38	140	175	2300	2650	93.64
Experiment 5.		Temperature : 23 ℃, Barometric Pressure : 29.87 in. Hg					
30	0.2	39	260	420	4600	3750	91.86
60	0.2	39	205	375	3800	3800	92.37
90	0.2	39	190	265	2950	3250	92.66
120	0.2	38	270	430	4500	4500	92.22

Untreated filter 2							
Experiment 1.		Temperature : 22 ℃, Barometric Pressure : 30.15 in. Hg					
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)		Control (PFU/mL)		Removal Eff. (%)
			Impinger-1	Impinger-2	Impinger-1	Impinger-2	
30	0.2	40	235	195	2050	1950	89.25
60	0.2	39	185	95	1050	3200	93.41
90	0.2	39	190	255	1600	2800	89.89
120	0.2	38	160	115	1900	1300	91.41
Experiment 2.		Temperature : 23 ℃, Barometric Pressure : 30.05in. Hg					
30	0.2	39	795	865	3200	7450	84.41
60	0.2	39	265	250	4300	5100	94.52
90	0.2	38	420	690	4900	5300	89.12
120	0.2	38	160	330	1750	3600	90.84
Experiment 3.		Temperature : 22 ℃, Barometric Pressure : 30.10in. Hg					
30	0.2	40	340	575	3350	5100	89.17
60	0.2	39	375	220	2450	3150	89.38
90	0.2	38	555	810	5900	12600	92.62
120	0.2	38	440	290	4000	5400	92.23
Experiment 4.		Temperature : 22 ℃, Barometric Pressure : 29.87 in. Hg					
30	0.2	39	1330	375	7450	9600	90.00
60	0.2	39	495	310	2450	5900	90.36
90	0.2	39	350	515	2200	5600	88.91
120	0.2	38	175	535	3200	5950	92.24
Experiment 5.		Temperature : 22 ℃, Barometric Pressure : 29.87 in. Hg					
30	0.2	40	820	660	1200	21100	93.36
60	0.2	39	575	645	1950	13100	91.89
90	0.2	40	280	255	2000	3250	89.81
120	0.2	38	80	335	1100	3700	91.35

Untreated filter 3							
Experiment 1.		Temperature : 23.5 ℃, Barometric Pressure : 30.21 in. Hg					
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)		Control (PFU/mL)		Removal Eff. (%)
			Impinger-1	Impinger-2	Impinger-1	Impinger-2	
30	0.2	39	300	150	4900	1450	92.91
60	0.2	39	75	190	4000	4000	96.69
90	0.2	38	315	480	5200	7750	93.86
120	0.2	38	640	570	16900	12050	95.82
Experiment 2.		Temperature : 23.5 ℃, Barometric Pressure : 30.21in. Hg					
30	0.2	40	380	1300	8400	7000	89.09
60	0.2	40	1100	1500	6850	13900	87.47
90	0.2	39	130	640	10000	3450	94.28
120	0.2	38	250	300	2850	3500	91.34
Experiment 3.		Temperature : 24 ℃, Barometric Pressure : 29.99in. Hg					
30	0.2	40	155	535	3600	2700	89.05
60	0.2	39	300	365	3100	4950	91.74
90	0.2	38	310	625	4950	4150	89.73
120	0.2	38	280	210	3000	2450	91.01
Experiment 4.		Temperature : 23 ℃, Barometric Pressure : 29.99 in. Hg					
30	0.2	39	630	250	4950	6100	92.04
60	0.2	39	345	365	3450	4900	91.50
90	0.2	39	250	425	4200	5000	92.66
120	0.2	38	225	435	3700	5450	92.79
Experiment 5.		Temperature : 23 ℃, Barometric Pressure : 29.99 in. Hg					
30	0.2	40	470	410	5200	8750	93.69
60	0.2	38	425	475	3950	6600	91.47
90	0.2	38	275	310	4000	3700	92.40
120	0.2	38	265	375	3600	5200	92.73

Appendix C. Shielding Effect Experiment Raw Data

Collection Experiment 1							
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)		Control (PFU/mL)		Removal Eff. (%)
			Impinger-1	Impinger-2	Impinger-1	Impinger-2	
0.l mL of viral stock		Temperature : 24 ℃, Barometric Pressure : 30.06 in. Hg					
30	0.2	39	12020	14485	454150	314100	96.56
0.5 mL of viral stock		Temperature : 24 ℃, Barometric Pressure : 30.06in. Hg					
30	0.2	40	32675	36110	526600	568800	93.45
1 mL of viral stock		Temperature : 24 ℃, Barometric Pressure : 30.06in. Hg					
30	0.4	38	1.0×10^{12}	1.5×10^{12}	1.1×10^{15}	9×10^{14}	99.82

Vortexing Experiment 1					
Extracted Microorganisms (PFU)			Relative Fraction		
1 min	5 min	10 min	1 min	5 min	10 min
0.l mL of viral stock					
6.1×10^{5}	1.9×10^{6}	2.5×10^{6}	0.04	0.13	0.17
1.7×10^{06}			0.11		
0.5 mL of viral stock					
3×10^{6}	6×10^{6}	6×10^{6}	0.17	0.28	0.30
5×10^{6}			0.25		
1 mL of viral stock					
8×10^{14}	9×10^{15}	9×10^{15}	0.02	0.22	0.22
6×10^{15}			0.15		

Collection Experiment 2							
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)		Control (PFU/mL)		Removal Eff. (%)
			Impinger-1	Impinger-2	Impinger-1	Impinger-2	
0.l mL of viral stock		Temperature : 23 ℃, Barometric Pressure : 30.10 in. Hg					
30	0.2	39	10050	11170	460000	440000	97.64
0.5 mL of viral stock		Temperature : 23 ℃, Barometric Pressure : 30.10in. Hg					
30	0.3	38	3×10^{7}	4 x 107	4×10^{9}	7×10^{8}	98.66
1 mL of viral stock		Temperature : 23 ℃, Barometric Pressure : 30.10in. Hg					
30	0.4	38	7×10^{11}	4×10^{11}	3 x 1013	4×10^{13}	98.45

Vortexing Experiment 2					
Extracted Microorganisms (PFU)			Relative Fraction		
1 min	5 min	10 min	1 min	5 min	10 min
0.l mL of viral stock					
1.8×10^6	1.9×10^6	1.0×10^6	0.10	0.11	0.23
2.6×10^6			0.15		
0.5 mL of viral stock					
4×10^{10}	4×10^{10}	6×10^{10}	0.4	0.4	0.6
5×10^{10}			0.5		
1 mL of viral stock					
2.2×10^{14}	1.7×10^{14}	1.8×10^{14}	0.16	0.12	0.13
1.9×10^{14}			0.14		

Appendix D. Pressure Drag Calculation

- Glass fiber filters (Millipore AP 15)
  - Air resistance at 10.5 fpm : 210 mm H_2O [64]

$$S_{drag} = \frac{\Delta P}{V_f} = \frac{8.27 in H_2O}{126 in/\min} = 0.0656 \quad in H_2O/(in/\min)$$

Pressure drop: $\Delta P = 210 mm H_2O \times \frac{0.001 m H_2O}{mm H_2O} \times \frac{39.37 in H_2O}{1 m H_2O} = 8.27 in H_2O$

Filter velocity: $V_f = 10.5 \frac{ft}{\min} \times \frac{12 in}{ft} = 126 in/\min$

- Filters for bacterial spores experiment
  - Air resistance at 15 Lpm : 3.0 in H_2O

$$S_{drag} = \frac{\Delta P}{V_f} = \frac{3.0 in H_2O}{335 in/\min} = 0.009 \quad in H_2O/(in/\min)$$

Filter velocity: $V_f = 14.2 \frac{cm}{\sec} \times \frac{0.3937 in}{cm} \times \frac{60 \sec}{\min} = 335 in/\min$

- Filters for viral experiment
  - Air resistance at 15 Lpm : 0.2 in H_2O

$$S_{drag} = \frac{\Delta P}{V_f} = \frac{0.2 in H_2O}{335 in/\min} = 0.0006 \ in H_2O/(in/\min)$$

Appendix E. Statistical Analysis of vortexing experiment results - Input data

	Vortexing time (min)				
Virus conc.	1	2	3	5	10
36	0.02778	0.13889	0.08333	0.19444	0.05556
66	0.04545	0.22727	0.45455	0.04545	0.21212
163	0.02454	0.11656	0.19632	0.05521	0.04908
294	0.39116	0.21769	0.17007	0.45578	0.30952
441	0.11565	0.15420	0.17687	0.16780	0.14059

- Results: Concentration

SUMMARY

Groups	Count	Sum	Average	Variance
36	5	0.5	0.1	0.00448
66	5	0.9848485	0.1969697	0.0283517
163	5	0.44171779	0.08834356	0.00479
294	5	1.5442177	0.3088435	0.0139838
441	5	0.75510204	0.15102041	0.00058

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P*-value	*F* crit
Between Groups	0.1596412	4	0.0399103	3.8241394	0.0181973	2.866081
Within Groups	0.2087283	20	0.0104364			
Total						

- Results: Vortexing time

SUMMARY

Groups	Count	Sum	Average	Variance
a	5	0.6045749	0.120915	0.0241788
b	5	0.8546081	0.1709216	0.0024058
c	5	1.0811366	0.2162273	0.0196331
d	5	0.9186965	0.1837393	0.0275043
e	5	0.7668699	0.153374	0.0121108

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P*-value	*F* crit
Between Groups	0.0250385	4	0.0062596	0.3646404	0.8308611	2.866081
Within Groups	0.343331	20	0.0171666			
Total						

Appendix F. Media Preparation for Plaque assay

- **Tryptone bottom agar**

  With gentle mixing, 1.0 g tryptone, 0.1 g yeast extract, 0.1 g glucose, 0.8 g NaCl, 0.022 g $CaCl_2$, and 1.5 g of Bacto-agar were added to a total volume of 100 mL of distilled water in a 250-mL flask. The mixed agar was autoclaved at 121 ^{o}C for 30 min. Fifteen-mL aliquots were pipeted aseptically into sterile 100 × 15 mm Petri dishes and the agar was allowed to harden. The inverted dishes were stored at 4 ^{o}C overnight and were warmed to room temperature for 1 hr before use. The agar was prepared one day prior to sample analysis.

- **Tryptone top agar**

  With gentle mixing, 1.0 g tryptone, 0.1 g yeast extract, 0.1 g glucose, 0.8 g NaCl, 0.022 g $CaCl_2$, and 0.5 g of Bacto-agar were added to a total volume of 100 mL of distilled water in a 250-mL flask. The mixed agar was autoclaved at 121 ^{o}C for 30 min one day prior to sample anaysis.

- **Tryptone broth**

  Tryptone (1.0 g), 0.1 g yeast extract, 0.1 g glucose, 0.8 g NaCl and 0.022 g $CaCl_2$ were added to a total volume of 100 mL of distilled water and autoclaved at 121 ^{o}C for 30 min.

- **Tryptone dilution tubes**

  Nine-mL aliquots of sterile tryptone broth were aseptically dispensed into 16 × 150-mm screw-capped test tubes that had been sterilized by autoclaving at 121 ^{o}C for 30 min.